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Online Conference

Plant breeding for the ‘Green Deal’

SAATGUT AUSTRIA

Vereinigung der Pflanzenzüchter
und Saatgutkaufleute Österreichs

# 72. Tagung

**22.-24. November 2021**
**Online Conference**

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*Agroinfiltration of Phytophthora infestans effector gene Avr4 on potato detached leaves. The resistance protein R4 in leaf tissue interacts with Avr4 from the vector Agrobacterium tumefaciens, which results in hypersensitive response (left) compared to the mock control of A. tumefaciens with an empty vector (right).*

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## Preface

In its 72-year long history, the Annual Conference of the Austrian Association of Plant Breeders and Seed Merchants was now held two consecutive years as an online conference due to COVID-19 restrictions. The topic of the conference from 22nd-24th November 2022 was *Plant breeding for the Green Deal*.

The conference was opened by a key-note lecture on *The European Green Deal and its farm to fork strateg*y by Georg Häusler from the European Commission, Directorate-General for Agriculture and Rural Development (AGRI). The European Green Deal was announced in December 2019 as a response to climate change, loss of biodiversity and environmental pollution, aiming to improve the well-being of people. The communication, press releases, highlights, actions, factsheets and other documents on this European priority can be retrieved at the official website of the European Union (https://ec.europa.eu/info/strategy/priorities-2019-2024/european-green-deal_en). The Farm to Fork strategy, which Mr. Häusler introduced in his lecture, is at the heart of the European Green Deal with the aim to make food production, food processing and distribution, and food consumption more sustainable, as well as to prevent food loss and waste (https://ec.europa.eu/food/horizontal-topics/farm-fork-strategy_de).

Plant breeding is considered a congenial partner to the European Union’s strategy towards more sustainable developments in agriculture and beyond. However, various scientists believe that the goals of Europe’s Farm to Fork strategy may be jeopardized by insisting on the current regulation of new plant breeding techniques and biotech innovations. Kai Purnhagen, Chair of Food Law at the University of Bayreuth, outlined in his lecture aspects of conflicts in the EU’s commitment to biotechnology and organic farming. Prof. Purnhagen’s arguments and ideas can be retrieved from several recent publications (*e.g.*, Purnhagen *et al.*, 2018; 2021; Eriksson *et al.*, 2019; Purnhagen & Wesseler, 2021; Wesseler *et al.*, 2022)

In the meantime, Russia’s invasion of Ukraine got worse the rising of global food prices which started already in fall 2020 with lower harvests and disrupted supply chains due to climate change and COVID-19, respectively. Especially dramatic is the rise of the vegetable oil price (FAO, 2022) as the Black Sea region is a crucial source for sunflower oil. Hence, voices rose to water down the objectives, targets and timeline of the Farm to Fork strategy for Europe’s food security (Bounds, 2022). A rather improper demand in view of 88 million tons of food waste per year (EUFIC, 2021; European Commission, https://ec.europa.eu/food/safety/food-waste_en). Therefore, the challenge is not so much an increase in production but in avoiding food waste. Moreover, significant amounts of grain are produced to feed swine and poultry although meat consumption in most European countries is already too high and associated with adverse health behaviours and characteristics (Richi *et al.*, 2015; Papier *et al.*, 2021). Intensive agriculture and livestock production ignoring animal welfare also leads to deforestation, loss of biodiversity, habitat fragmentation and pollution. Besides increasing urbanization and global connectedness, it is these environmental factors which in return are drivers of increasing human-animal contacts and accelerated transmission rates of zoonotic diseases such as COVID-19 (Mishra *et al.*, 2021; Holmes, 2022). Europe’s Farm to Fork strategy might be ambitious from today’s viewpoint but inevitable in order to stop the growth of badlands caused by human greed. Overexpansion, climate change, environmental degradation and wrong leadership led to the collapse of Ancient Egypt, Carthage, Classical Greek, the Roman Empire, Mesoamerican and many other societies in history (see *e.g*. Diamond, 2005). Today we are visiting the ruins of these vanished cultures, we are impressed by their size and beauty, they stimulate our fantasy, but have we learned their lessons?

Heinrich Grausgruber

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72. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
22.-24. November 2021, Online Conference, Raumberg-Gumpenstein, Österreich

# Nachruf: Josef Rath (1928-2022)

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Nach einem erfüllten Leben ist Hr. Ing. Josef Rath - langjähriger Züchter der Saatzucht Gleisdorf - am 28. Jänner 2022 im Lebensalter von 94 Jahren wohlvorbereitet im Kreise seiner Familie verstorben.

Josef Rath wurde am 30. Juni 1928 in Hinterwald bei Hirnsdorf geboren. Nach der Pflichtschule besuchte Josef Rath das Francisco Josephinum in Weinzierl, Wieselburg. Von dort wurde er im Jahre 1945 zum Arbeits- und Kriegsdienst einberufen. Nach seiner Rückkehr setzte er im Jahre 1946 sein Studium am Francisco Josephinum fort. Nach erfolgreichem Abschluss wurde Josef Rath im November 1947 als Assistent an der Saatzucht- und Versuchsanstalt, damals in Kornberg, von der Landeskammer Steiermark eingestellt. Im Jahre 1956/57 übersiedelte die Saatzucht in das neu errichtete Gebäude am Tieberhof in Gleisdorf.

In seiner über 40 Jahre dauernden züchterischen Tätigkeit wurde eine Vielzahl an Kulturarten von ihm bearbeitet. Seine Aufgabe bestand darin, bei den Kulturarten Mais, Ölkürbis, Ackerbohne, Käferbohne und Rispenhirse neue Sorten mit verbesserten agronomischen Eigenschaften und höherer Ertragsleistung zu entwickeln. Seine erfolgreiche Arbeit wird dadurch bestätigt, dass zahlreiche Sorten aus seiner Zeit noch heute im In- und Ausland erfolgreich angebaut werden, wie zum Beispiel die Ölkürbissorte ‘Gleisdorfer’, die Rispenhirse ‘Kornberger Mittelfrühe’, die Käferbohne ‘Bonela’ oder die Buchweizensorte ‘Bamby’. Neben dem Akquirieren von passenden Getreidesorten für den südlichen Anbauraum war er auch in der Tabaksortenentwicklung für die damalige österreichische Tabakregie tätig.

Sein Engagement, seine Gewissenhaftigkeit und seine Menschlichkeit waren stets vorbildhaft. Gerne diskutierte er auch über politische und gesellschaftliche Entwicklungen. 1985 wurde er mit der Prokura der Saatzucht Gleisdorf betraut. Sein geistiges Erbe, sein Wissen, seine begonnenen Selektionen hat er mit Freunde an viele Praktikanten, aber vor allem an seine Nachfolgerin Frau DI Johanna Winkler weitergegeben. Viele Jahre danach war er stolz auf die erfolgreiche Tätigkeit von Frau Winkler und auf die weitere Entwicklung der Saatzucht Gleisdorf. Der enge Kontakt zu den Bäuerinnen und Bauern in der Region, den Institutionen und Partnern im In- und Ausland brachte ihm viel Sympathie ein.

Josef Rath (rechts) gemeinsam mit Frau Dr. Helga Pillovi und Herrn Fuchs beim Messen der Kornfeuchtigkeit im Maiszuchtgarten der Saatzucht Gleisdorf im Jahr 1959.

Als Mitarbeiter der Landeskammer Steiermark wurde er mit der Kammermedaille in Bronze und Silber ausgezeichnet und im Jahre 1989 trat er in die Pension über. Er war stets ein gern gesehener Gast im Hause der Saatzucht Gleisdorf und eine große Freude bereiteten ihm die Feiern zum 80-jährigen und zum 90-jährigen Geburtstag.

Sein Leben wurde geprägt durch die Liebe zu seiner Familie, durch die Pflanzenzüchtung an der Saatzucht Gleisdorf, aber auch durch die vielfältigen Tätigkeiten in seiner Freizeit bis in hohe Alter. Seien es die Aufgaben im Alpenverein, der stets gepflegte Garten rund ums Haus und die langjährigen Wetterbeobachtungen für die Zentralanstalt für Meteorologie und Geodynamik (ZAMG). Für die jahrelangen Meldungen der Wetterdaten an die ZAMG wurde Josef Rath 2001 mit dem Goldenen Ehrenzeichen der Republik ausgezeichnet, für die verdienstvolle Arbeit im Alpenverein 2005 mit dem Landessportabzeichen in Gold.

Wir werden Ing. Josef Rath stets ein ehrendes Andenken bewahren.

Posch J (2022) Nachruf: Josef Rath (1928-2022). In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 72. Jahrestagung 2021, 22-24 November, Online Conference, Raumberg-Gumpenstein, p 1. University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-96-1

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# The Nagoya Protocol and its implementation in the European Union

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## Abstract

The *Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization*, usually referred to as Nagoya Protocol (NP), is a supplementary agreement adopted in October 2010 by the Convention on Biological Diversity (CBD) to enhance the implementation effectiveness of its basic objectives. It specifies the access and benefit sharing obligations for countries providing and using genetic resources, making them legally-binding for the parties. With this protocol, the CBD aims at preventing biopiracy as well as ensuring the participation of the countries of origin and indigenous or local communities in the benefits arising from the use of genetic resources. As of January 1st, 2022, 132 countries worldwide are parties to the NP, including the European Union (EU).

The main pillar of the NP is the principle of sovereignty, which states that every country holds rights over animals, plants and genetic resources found within its borders, including the traditional knowledge associated with them. Potential users from a foreign country must respect this sovereign right by accessing such resources only after allowance by the providing country and sharing the benefits arising from their utilization (*e.g.*, research and development). All parties are required to take measures related to monitoring and communication and must establish at least one Competent National Authority (CNA) and Official Checkpoints. In addition, the NP establishes a system named ABS Clearing-House that facilitates the necessary information exchange between users, providers, and governments. The ABS Clearing-House monitors the resources leaving the provider country and the milestones related to their abroad utilization. Before accessing a foreign genetic resource, a user must obtain prior informed consent (PIC) from the provider country by outlining the nature and the intended goal of the genetic resource utilization. Afterwards, mutually agreed terms (MAT) must be established to define the benefits for the resource provider and further contractual issues. Once these documents are agreed upon, the CNA issues a permit and registers it on the ABS Clearing-House, creating a so-called internationally recognized certificate of compliance (IRCC) that allows official access to the foreign genetic resource. Once a new product is developed from its utilization, the user must contact his national Official Checkpoint, which will inform the CNA of the provider country. The latter checks if all requirements that had been agreed upon are met and authorizes the final procedures.

Since every party needs to translate the NP provisions into domestic law, the EU fulfilled these requirements by developing three pieces of legislation that describe the proceedings at different levels. *Regulation (EU) No 511/2014 on compliance measures for users from the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization in the Union* (commonly known as ABS Regulation) harmonizes the establishment of NP procedures at EU level. The ABS Regulation is complemented by Commission Implementing Regulation (EU) 2015/1866, which lays down detailed rules for its implementation, and the Guidance document on the scope of application and core obligations.

The core obligation for users under the NP is the exercise of due diligence to ascertain that their utilization of the genetic resources respects the legal requirements. This obligation applies not only to individuals (such as researchers), but also to organizations, enterprises, and companies of all sizes, if these utilize genetic resources or traditional knowledge associated with genetic resources. The necessary steps to undertake to ensure that due diligence is met are shown in Fig. 1.

If the provider country is a party to the NP, the user must check whether there are national applicable access and benefit-sharing legislations and/or regulatory requirements. If such information cannot be found on the ABS Clearing-House, but there are reasons to believe that it may nonetheless exist, the provider country's NFP should be contacted directly. Cases in which the genetic resources are obtained indirectly, are not excluded from due diligence duties. Where such conditions apply, the user should ensure that the PIC was obtained and the MAT were established by the intermediary when the resources were originally accessed. Any information relevant for access and benefit-sharing must be retained for a 20-year period after the end of of utilization.

A common exemption to the NP is represented by the so-called Seed Treaty (the International Treaty on Plant Genetic Resources for Food and Agriculture, ITPGRFA): the 64 crops included in its Multilateral System are not subjected to the provisions of the NP if accessed from and by countries that are parties to the Seed Treaty and utilized for the purposes of the Seed Treaty (which solely includes food and agricultural purposes).

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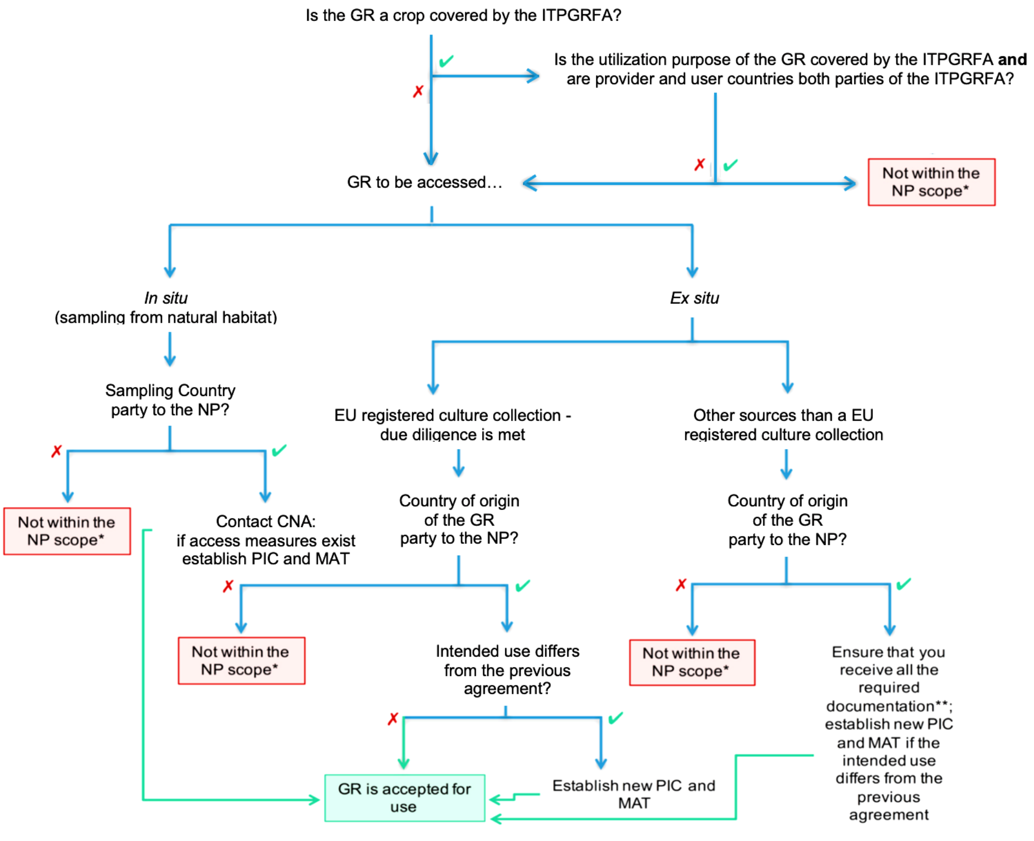

*Contact NFP, CNA or National Authorities of the country in order to comply with national legislations; **PIC,MAT, IRCC, MTA

**Figure 1** Nagoya Protocol (NP) decision tree for accessing genetic resources (GR) in accordance with the scope of the ABS Regulation (adapted from Martins *et al.* 2020). CNA: Competent National Authorities; ITPGRFA: International Treaty on Plant Genetic Resources for Food and Agriculture; IRCC: International Recognized Certificate of Compliance; MTA: Material Transfer Agreement; MAT: Mutually Agreed Terms; NFP: National Focal Point; PIC: Prior Informed Consent

To facilitate the users´ exercise of due diligence the EU released DECLARE, a web-based tool that supports them in submitting the required documentation to the relevant authorities and assists the Member States in transmitting the declarations to the ABS Clearing-House. The use of DECLARE (https://webgate.ec.europa.eu/declare/) is advisable but not mandatory, with national exceptions for users from France and Spain.

## Keywords

ABS Clearing-House · benefit sharing · Convention on Biological Diversity · genetic resources · prior informed consent

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# Adapting to a changing climate: first steps towards assessing a variety's climate-fitness in VCU trials

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## Abstract

To adapt to a changing climate and guarantee food security in Austria, improved crop varieties are needed. Ideally, these crops possess a high climate-fitness defined by a high yield stability even under changing weather conditions. However, to which extent can this climate-fitness be reliably tested in VCU trials? As part of the project KLIMAFIT 2, AGES conducts preliminary trials to check for the possibility of representing the performance of new varieties with regard to drought stress tolerance in the Descriptive List of Varieties. For this purpose, a soybean (*Glycine max*) field trial was established in 2021 to assess the possibility of screening new varieties and breeding lines with regard to their reaction to drought stress. In total 28 different soybean genotypes were grown under irrigated and non-irrigated conditions in Fuchsenbigl and assessed repeatedly and regularly in regard to plant height, above ground biomass as well as closing of rows over the course of the growing season. Grain yield for each plot was determined at harvest. All assessed parameters show significant differences between irrigated and non-irrigated groups, while the respective genotypes also display significant differences. In the field trial, varieties and breeding lines were identified which achieved a high performance in assessed traits both under drought stress as well as under normal conditions. The results of the preliminary trial provide useful first insights on the methodology of how to assess and display the performance of new crop varieties and breeding lines with regard to drought stress tolerance.

## Keywords

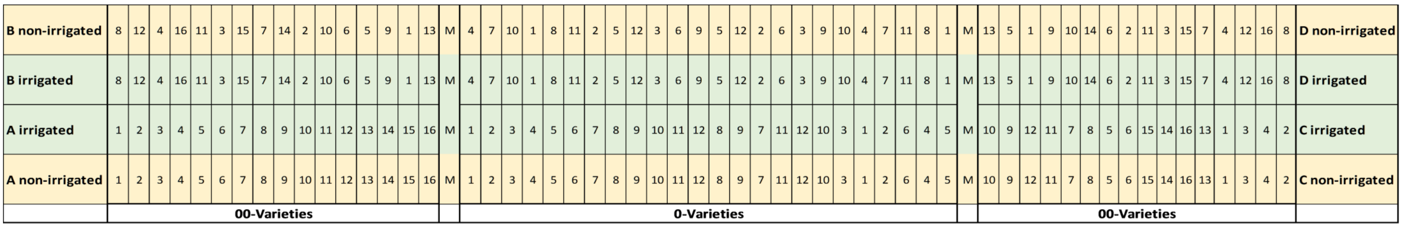
Abiotic stress · adaptation · drought · food security · *Glycine max* · soybean · yield stability

## Introduction

The effects of climate change on future climatic conditions in Austria are complex. However, forecasts predict a significant increase in heat days and a change in precipitation patterns, resulting in less rainfall coupled with longer periods of heat and drought in the summer months, while winter months will probably experience an increase in precipitation. The occurrence of extreme weather events is also likely to increase (Formayer *et al.*, 2009). Especially the arable farming areas in the eastern parts of Austria will have to adjust to longer periods of drought in the future, which will have a negative impact on yield and soil fertility (Strauss *et al.*, 2013; Haslmayer *et al.*, 2018). Climate change thus poses an existential threat to food security in Austria, and changing climate conditions demand for adapted crop varieties. Hence, improved, high-yielding, climate-fit varieties will play a vital part in ensuring food security. Ideally, these climate-fit varieties display a high degree of eco-stability, *i.e.* a high, reliable yield stability, even under changing weather conditions like prolonged drought stress.

Including the climate-fitness of a variety in its variety description would be beneficial for end users like farmers, when choosing which variety to grow in the field. However, to which extent can this climate-fitness be tested reliably, standardized and with high practicability during the value of cultivation and use (VCU) trials? In addition, which phenotypic parameters are most target-oriented for this issue? The Austrian breeding community in cooperation with Saatgut Austria and the AGES work together in the project KLIMAFIT 2 to develop climate fit varieties for Austria with a special focus on heat and drought stress tolerance. Additionally, as part of this project, the AGES conducts preliminary trials to check for the possibility of describing the performance of new varieties with regard to drought stress tolerance in the Descriptive List of Varieties. For this purpose, a soybean (*Glycine max*) field trial was established in 2021 to assess the possibility of screening new varieties and breeding lines with respect to their reaction to drought stress.

Von Gehren P, Bomers S, Ribarits A, Mottl K, Mechtler K, Flamm C, Brandstetter A (2022) Adapting to a changing climate: first steps towards assessing a variety's climate-fitness in VCU trials. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 72. Jahrestagung 2021, 22-24 November, Raumberg-Gumpenstein, pp 5-8. University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-96-1



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| **B non-irrigated** | 8 | 12 | 4 | 16 | 11 | 3 | 15 | 7 | 14 | 2 | 10 | 6 | 5 | 9 | 1 | 13 | M | 4 | 7 | 10 | 1 | 8 | 11 | 2 | 5 | 12 | 3 | 6 | 9 | 5 | 12 | 2 | 6 | 3 | 9 | 10 | 4 | 7 | 11 | 8 | 1 | M | 13 | 5 | 1 | 9 | 10 | 14 | 6 | 2 | 11 | 3 | 15 | 7 | 4 | 12 | 16 | 8 | **D non-irrigated** |
| **B irrigated** | 8 | 12 | 4 | 16 | 11 | 3 | 15 | 7 | 14 | 2 | 10 | 6 | 5 | 9 | 1 | 13 | M | 4 | 7 | 10 | 1 | 8 | 11 | 2 | 5 | 12 | 3 | 6 | 9 | 5 | 12 | 2 | 6 | 3 | 9 | 10 | 4 | 7 | 11 | 8 | 1 | M | 13 | 5 | 1 | 9 | 10 | 14 | 6 | 2 | 11 | 3 | 15 | 7 | 4 | 12 | 16 | 8 | **D irrigated** |
| **A irrigated** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | M | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 8 | 9 | 7 | 11 | 12 | 10 | 3 | 1 | 2 | 6 | 4 | 5 | M | 10 | 9 | 12 | 11 | 7 | 8 | 5 | 6 | 15 | 14 | 16 | 13 | 1 | 3 | 4 | 2 | **C irrigated** |
| **A non-irrigated** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | M | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 8 | 9 | 7 | 11 | 12 | 10 | 3 | 1 | 2 | 6 | 4 | 5 | M | 10 | 9 | 12 | 11 | 7 | 8 | 5 | 6 | 15 | 14 | 16 | 13 | 1 | 3 | 4 | 2 | **C non-irrigated** |
| | **00-Varieties** | | | | | | | | | | | | | | | | | **0-Varieties** | | | | | | | | | | | | | | | | | | | | | | | | | **00-Varieties** | | | | | | | | | | | | | | | | |

**Figure 1** Experimental plan of the soybean field trial at the AGES trial site in Fuchsenbigl.

## Material and methods

The soybean field trial was set up on 28th April 2021 at the AGES trial site in Fuchsenbigl (48°11'34"N, 16°44'59"E). The trial site is located at 143 m a.s.l. and is characterized by a Pannonian climate, with an average precipitation of 577 mm per year and an average annual mean temperature of 11.3°C (AGES, 2021).

A total of 28 registered varieties and VCU candidates (from now on referred to only as varieties) were selected for cultivation. Out of these, 16 varieties belonged to maturity group 00 (early maturity, especially suitable for the Pannonian region), and 12 to maturity group 0 (medium to late maturity, to be grown in favorable locations). All 28 varieties were grown under irrigated and non-irrigated conditions, in four replications each, resulting in a total of 224 plots. Plot size was 13 $m^2$ with 4 rows per plot spaced 40 cm apart. The two maturity groups were separated from each other by border plots. To assess the behavior of the varieties under drought stress and normal conditions, half of the plots were irrigated during dry periods, while the other half was not irrigated at all (Fig. 1). The respective plots were irrigated by a mobile sprinkler system on 8th and 21st June, and on 28th July with 30 $mm \cdot m^{-2}$ each.

Plant height (cm), closing of rows (1 to 9 scale) and above ground biomass (open-end scale) were assessed for each plot on eight dates (6th, 9th, 12th, 16th, 19th, 23rd, 26th and 30th July). Plant height was recorded by measuring the height of four representative plants per plot. On each date, each plot was assigned a grade ranging from 1 (no visible closing of rows) to 9 (complete closing of rows, no soil visible between the rows) to track the closing of rows. Above ground biomass was graded on an open-end scale, starting from 1 (very little above ground biomass visible), and given in comparison to the other cultivated plots. The score was adjusted at each date based on the last score, so that there was a continuous increase in the scores over the entire trial period. Plots were harvested by a plot combine on 19th October. Grain yield ($dt \cdot ha^{-1}$) was determined on a moisture content of 13%.

## Results and discussion

Emergence of the crop was delayed by an unusually cold, foggy and wet May, in which no artificial irrigation was needed. Contrary to this, plenty of sunshine and many hot days were recorded for June. As a result, irrigation was provided to the respective plots. Already on the first evaluation date (6th July), a positive effect of the irrigation treatment on both plant height as well as above ground biomass became evident (Fig. 2). July was initially very hot and dry. In mid-July, heavy rainfall set in, from which the varieties of both treatments benefited.

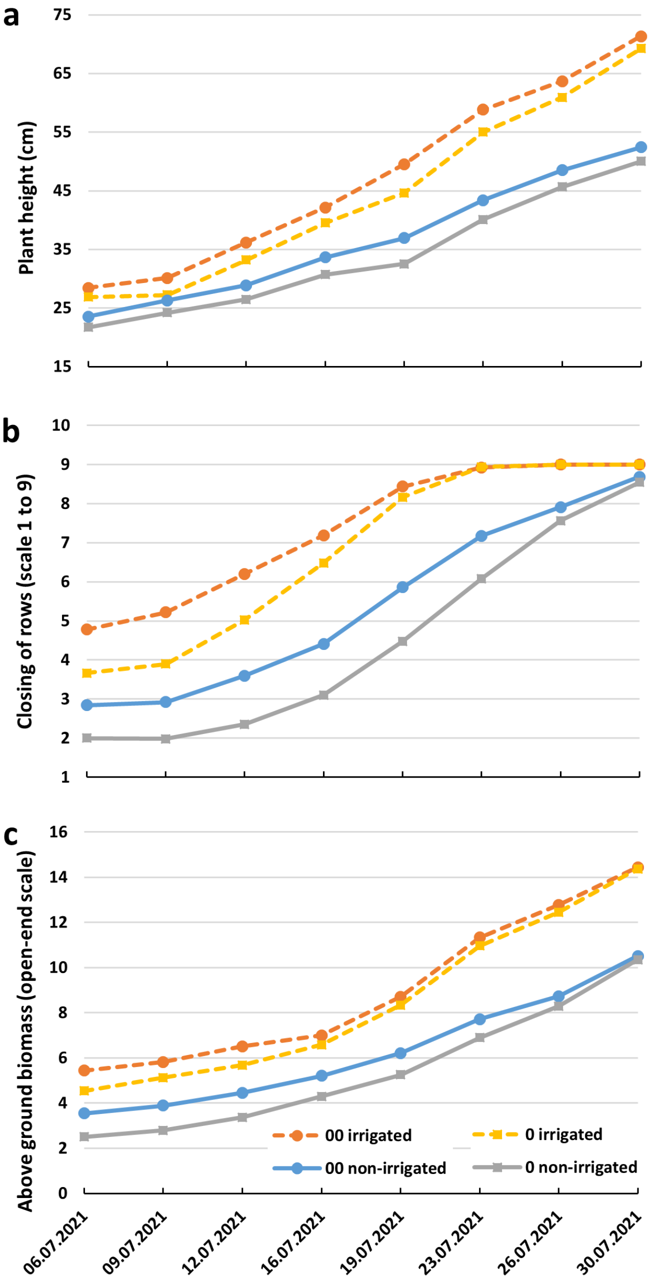

**Figure 2** Performance of soybean varieties of maturity group 0 and maturity group 00 in an irrigated and non-irrigated trial in Fuchsenbigl: **a** plant height; **b** closing of rows; **c** above ground biomass.

Over the course of the experiment, non-irrigated plots were unable to catch up with the original growth advantage of the irrigated plots, especially as a drought phase set in again by end of July. Here, the irrigated varieties benefited from the irrigation at the

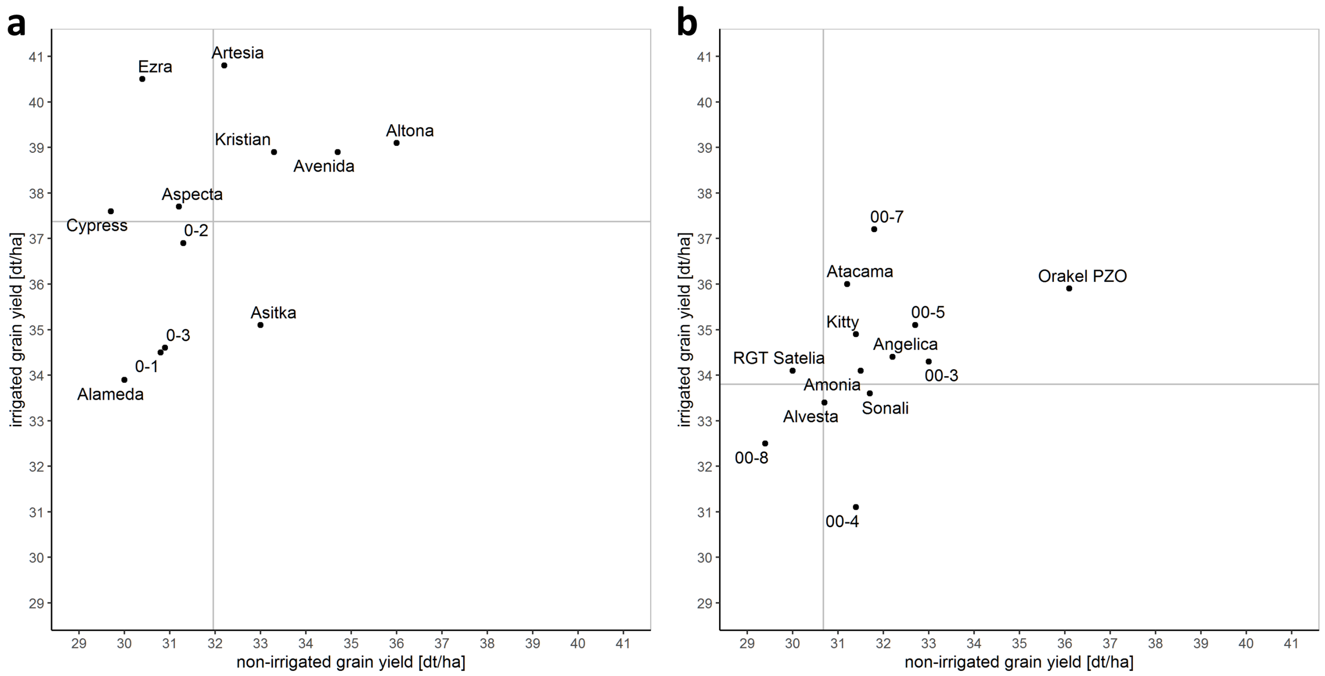

**Figure 3** Yield performance of soybean varieties (**a** maturity group 0; **b** maturity group 00): grain yield under irrigation plotted against grain yield under rainfed conditions in Fuchsenbigl 2021.

end of July. As a result, the advantage of irrigation on plant development became evident both in terms of plant height, where difference between irrigated and non-irrigated plots increased with proceeding growth period, as well as above ground biomass and closing of rows (Fig. 2). All assessed parameters showed significant differences between the irrigated and non-irrigated treatment, *e.g*. difference in final plant height was on average 20 cm, difference for above ground biomass was on average 4 grades. Closing of rows occurred approximately 7 to 10 days earlier in the irrigated plots. Compared to the irrigation treatment, the maturity groups were less affecting plant height and above ground biomass.

As we define a climate-fit variety as a variety that achieves high, reliable yield stability, even under changing weather conditions, those varieties which performed well under both drought stress (*i.e*. non-irrigated trial) as well as under irrigated conditions could be deemed climate-fit. In our experiment, the varieties displayed significant differences for all assessed parameters. Pronounced differences between varieties were also obvious in regard to grain yield. In both maturity groups, varieties were identified that performed superior in the assessed traits under drought conditions as well as under normal conditions. Their performance in these traits was positively correlated with grain yield. Consequently, a high climate-fitness could be presented descriptively by plotting the irrigated vs. the non-irrigated grain yields of the test entries in a scatter plot. Varieties with high grain yields under both conditions are then located in the top right corner (Fig. 3).

The desribed experimental set up as well as its presentation of results could be one potential way of assessing a variety's drought tolerance and ultimately lead to including the description of the climate-fitness of a variety in the Descriptive List of Varieties, providing additional beneficial information to end users. While the first results of the preliminary trials are promising, some limitations have to be considered. First, the results are based on a one-year trial and need to be confirmed across different environmental conditions, *e.g*. growing seasons with increased precipitation with no relevant drought stress. Secondly, it is still unclear which traits best describe the climate-fitness. Should the focus be mostly on grain yield or are other factors of higher importance for the end user? In this case, how do other variety characteristics differentiate under stress conditions? Finally, what is the best way to present the results with focus on its target group? By now, the preliminary trial can be the basis for an extended discussion of these questions as well as provide useful first insights on the methodology how to best assess and display the performance of new crop varieties with regard to drought stress in VCU trials.

## Acknowledgements

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72. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
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# Sensing approaches for integrating high throughput phenotyping with genomics: Exemplar

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## Abstract

High throughput phenotyping is a major bottleneck in potato especially when targeting phenotype-genotype association studies, with traditional phenotyping methodologies having high-resource requirements and tend to be qualitative in nature. To help alleviate many of these challenges, remote sensing techniques have been incorporated in agriculture over the last decade, and now allow the assessment of several key potato traits *e.g*. canopy grow, tuber shape, providing the needed quantitative data for QTL identification. In this paper we highlight how point clouds constructed utilizing captured UAV RGB-images (using the structure from motion approach), wherefrom individual plots were 3D surface modelled, provide canopy trait data, over the growing season. We provide two potato varieties ('Eclipse' and 'Robijn') as exemplars. This data permits the evaluation of plant physiological age (*e.g*. maturity), while also allowing the evaluation of individual growth patterns of each variety. It also exemplifies how the traditionally used plant height (which is the canopies maximum height) could lead to erroneous conclusions of plant physiological status, with mean height (resulting from the averaging of hundreds of points across the canopy) providing a more holistic approach.

## Introduction

In the last few decades, there has been tremendous advances in the fields of genomics, making it possible to now create complete genetic profiles in a fraction of the time and cost. Yet, despite the existence of these newly developed tools, technologies and the publication of the complete genome sequence of potato (*Solanum tuberosum* L.) (The Potato Genome Sequencing Consortium, 2011), there remains many key traits for which very little is known of the associated genetic mechanisms. There are many factors responsible for this, on the one side, many of the key traits (*e.g*. yield, growth, maturity) are complex traits that are the result of several interconnected quantitative trait loci (QTL) and, on the other side, many of the previously developed tools for diploids do not work as effectively due to the potatoes tetraploid nature (Ortiz, 2020).

The challenge to create accurate phenotype-genotype associations is further compounded by the low availability of high-quality phenotypical datasets. Which in itself is a symptom of the qualitative nature and the high resource intensity of traditional phenotyping methods (*e.g*. measuring tuber size using callipers, defining potato maturity in few unspecified categories, etc.) (Santini *et al*., 2000; Prashar *et al*., 2014; Hara-Skrzypiec *et al*., 2018). The high resource intensity of traditional phenotyping has impacted research over years either limiting the research to small number of varieties being assessed and/or to controlled environment settings (*e.g*. greenhouses), which may not translate to field conditions. If done in field condition, the resource intensity frequently limits the surveys/assessments to small sample plots in limited geographical area that may not represent the full scope of possible phenotypical outcomes (Anithakumari *et al*., 2011; Khan *et al*., 2015).

Remote sensing techniques in agriculture have become more popular in the last decade to help alleviate many of the challenges above (Prashar *et al*., 2013; Yang *et al*., 2017; de Jesus Colwell *et al*., 2021). Sensing approaches used for crop trait phenotyping and crop monitoring include satellite-based systems, manned aircraft, or unmanned aerial vehicles (UAVs) linked systems and tractor mounted sensing tools. Of all of these, UAV has shown some of the greatest potential, due in part to the increase accuracy and affordability of UAV's and its capacity to monitor relatively large agricultural fields with high spatial resolution in a short amount of time (Matese *et al*., 2015; Yang *et al*., 2017; de Jesus Colwell *et al*., 2021). The extent of information that one is able to gather with UAV's depends on the sensors used (Yang *et al*., 2017). The use of simple RGB sensors not only allows visual assessment of the sampled areas but can also allow the assessment of traits influencing plant/canopy development, such as ground cover and plant health. These images can be used to construct points cloud dataset from which plant height, and 3D plant structure data can be extracted. The inclusion of Near-Infrared (NIR) sensors allows estimation of various vegetation indexes that can be used to expand the above measurements to estimate biomass, nutrient stress and disease detection while thermal sensors are useful for understanding stress linked to water status and root based diseases (Yang *et al*., 2017). The combination of these different sensors allows the evaluation of several traits in an expedient and less

De Jesus Colwell F, Souter J, Prashar A (2022) Sensing approaches for integrating high throughput phenotyping with genomics: Exemplar. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 72. Jahrestagung 2021, 22-24 November, Raumberg-Gumpenstein, pp 9-11. University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-96-1

biased manner with the added potential of enabling the creation of accurate growth and development models through frequent data collection points (Prashar *et al*., 2013; Yang *et al*., 2017; de Jesus Colwell *et al*., 2021).

This paper highlights how the point cloud data (constructed from UAV captured RGB images) can help us evaluate the canopy growth development over time and how it can be used to identify relevant QTL's, related to plant height specifically.

## Materials and methods

The data used in this paper comes from a large study dataset that was performed at Nafferton Farm, Newcastle University, in 2018 (de Jesus Colwell *et al*., 2021). This study involves data collection on 297 varieties of potato, which form a part of a tetraploid variety association panel (Sharma *et al*., 2018). The experimental design consisted of two replicate blocks under two management systems (organic and conventional), making a total of 4 blocks. Plants were grown from early May to mid-September with UAV images captured at 33, 62, 95 and 117 days after planting in 2018 using structure from motion concept. Captured images were subsequently processed and point clouds generated in Trimble Business Center (Trimble, Sunnyvale, CA, USA). From the point cloud data, individual plots were 3D surface modelled, from which plant height and canopy characteristics data was obtained. A full detailed account of the methodological approach used is available in de Jesus Colwell *et al.* (2021).

## Results and discussion

The creation of ground surface models in the UAV datasets and its difference from the canopy surface models allow us to predict the canopy characteristics. These canopy characteristics data combi-

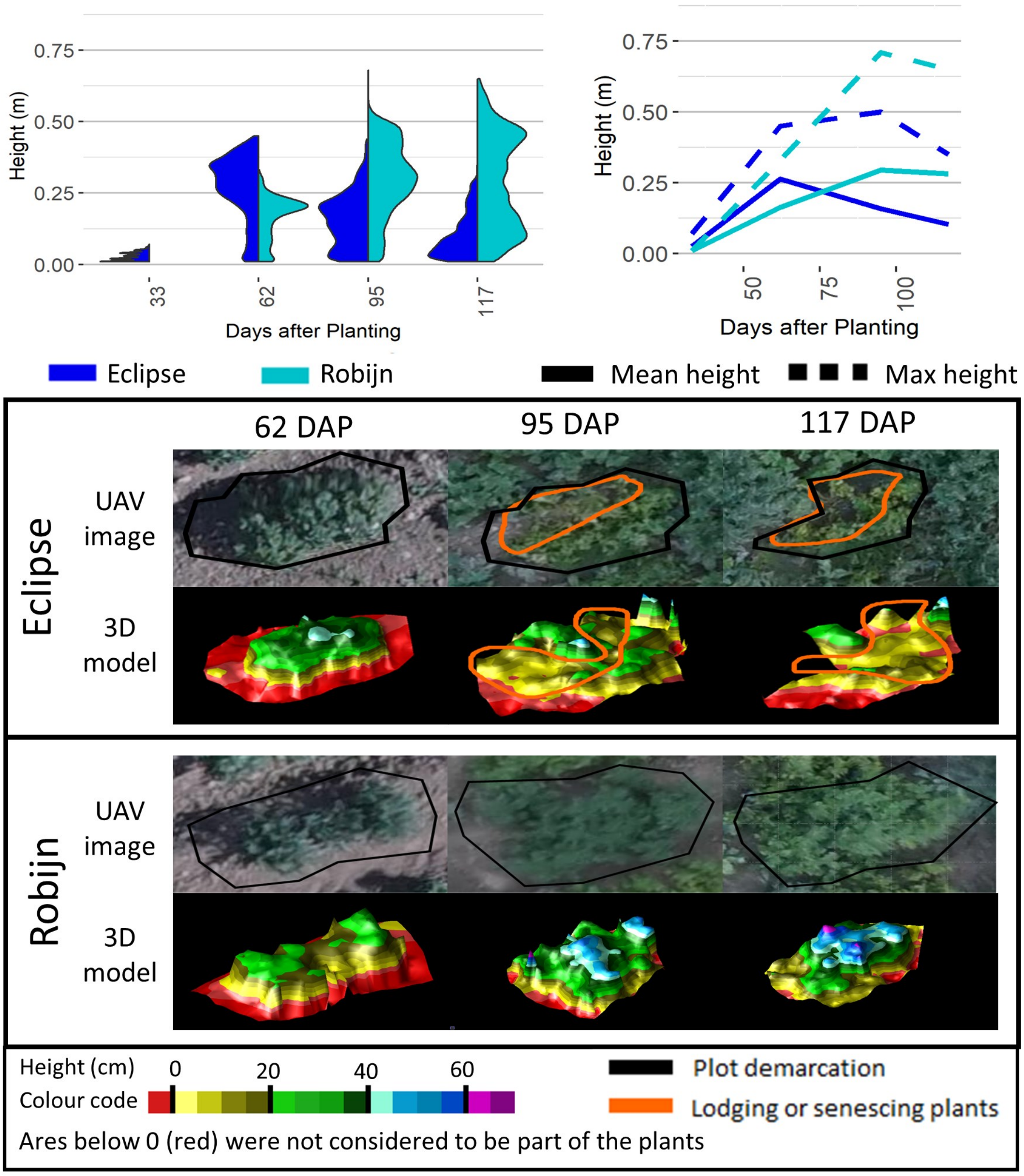

**Figure 1** Change in canopy profile data (height distribution of the canopy—violin plot, average and maximum height of canopy, solid and dashed line respectively) of 3-plant-plots of Eclipse and Robjin potato varieties over the growing season, accompanied with the corresponding UAV orthomosaic image and 3D models generated at 62, 95 and 117 days after planting.

ned at different temporal points helped generate growth curves or 3D growth models for different varieties used in this experiment. Fig. 1 shows models for two varieties (‘Eclipse’ and ‘Robijn’) and depicts the change in height of two 3-plant-plots for these varieties over the growing season. It is evident that these two varieties present distinct growth patterns, with the ‘Eclipse’ plants reaching peak size at around 62 days after planting (DAP), followed by a continuous decline of plant vigour until 117 DAP, as highlighted by the shift in the plant height profile to lower heights and the decrease in average height. Variety ‘Robijn’ grew at a slightly lower rate than ‘Eclipse’, but by 95 DAP, ‘Robijn’ surpassed the peak height of ‘Eclipse’, with ‘Robijn’ showing little to no loss of plant vigour by 117 DAP. This matches previously reported plant maturity, with ‘Eclipse’ being classified as a very early variety and ‘Robijn’ as a very late maturing variety (ECPD, 2021).

This data also highlights how the use of maximum height of the canopy could lead to erroneous conclusion. In ‘Robijn’ the maximum height and the mean height present relatively similar patterns, while in the case of ‘Eclipse’, maximum height was at 95 DAP, although there are clear signs of loss of plant vigour at this point. Therefore, evaluations at different physiological age (based on maturity) for different varieties is very crucial. Traditionally, most of the genetic studies linked with QTL mapping and association studies have used maximum height datasets and those too using single scale measurements. The data in Fig. 1 and point cloud data approach also highlight the importance of having mean data calculations based on hundreds of different points instead of a single measurement, which will help in decreasing the error mean in association studies.

**Keywords**

Canopy profile · remote sensing · *Solanum tuberosum*

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72. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
22.-24. November 2021, Online Conference, Raumberg-Gumpenstein, Österreich

# Development of a reliable field-testing methodology for *Fusarium langsethiae* resistance in oats confirms *F. langsethiae*-specific susceptibility in certain genotypes with otherwise good FHB resistance

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## Abstract

Fusarium head blight (FHB) is a serious challenge for the production of oats and other cereals in Norway. The challenges in oats relate to problems with both high mycotoxin levels and reduced germination ability of seeds. The *Fusarium* pathogens *F. graminearum* and *F. langsethiae* are of particular concern in Norway, causing contamination with DON and HT2+T2 mycotoxins, respectively. FHB resistance in cereals is generally known to be *Fusarium* pathogen non-specific. However, data from naturally infected field trials in Norway has shown occasionally high HT2+T2 levels in the oat variety ‘Odal’ which has a high level of resistance to *F. graminearum*.

In the SafeOats project (2016-2020) we developed a field-testing methodology for *F. langsethiae* in oats. Using spray inoculation at three different growth stages (heading, mid-anthesis and early grainfilling) in experiments over four years with six oat cultivars we identified mid-anthesis to be the most susceptible stage for infection. This is in line with results for other Fusarium pathogens. To investigate the resistance levels in a broader set of germplasm, 33 cultivars and breeding lines were tested in five field trials with the newly developed testing methodology based on spray-inoculation with *F. langsethiae* at anthesis in combination with slight mist irrigation at night. The same set of cultivars were also tested for resistance to *F. graminearum* in four separate field trials with our established grain spawn inoculation method that is routinely used for resistance testing. Mycotoxin data from the inoculated nurseries were compared with data from naturally infected field trials.

Cultivar rankings for the two Fusarium pathogens followed closely the available data from naturally infected fields confirming the reliability of the field-testing methodology. In general, resistance and susceptibility of the cultivars to the two *Fusarium* pathogens were correlated, except for a few genotypes including ‘Odal’, that showed a dramatically higher susceptibility to *F. langsethiae* than expected based on the *F. graminearum* data (Fig. 1). We hypothesize that the high susceptibility of cultivar ‘Odal’ could be due to a susceptibility gene that is specific to *F. langsethiae*. A mapping population is currently being developed to investigate the genetics of this putative *F. langsethiae*-specific susceptibility and to develop molecular markers that can be used in resistance breeding.

## Keywords

*Avena sativa* · deoxynivalenol (DON) · Fusarium head blight · HT2+T2 toxin · mycotoxin

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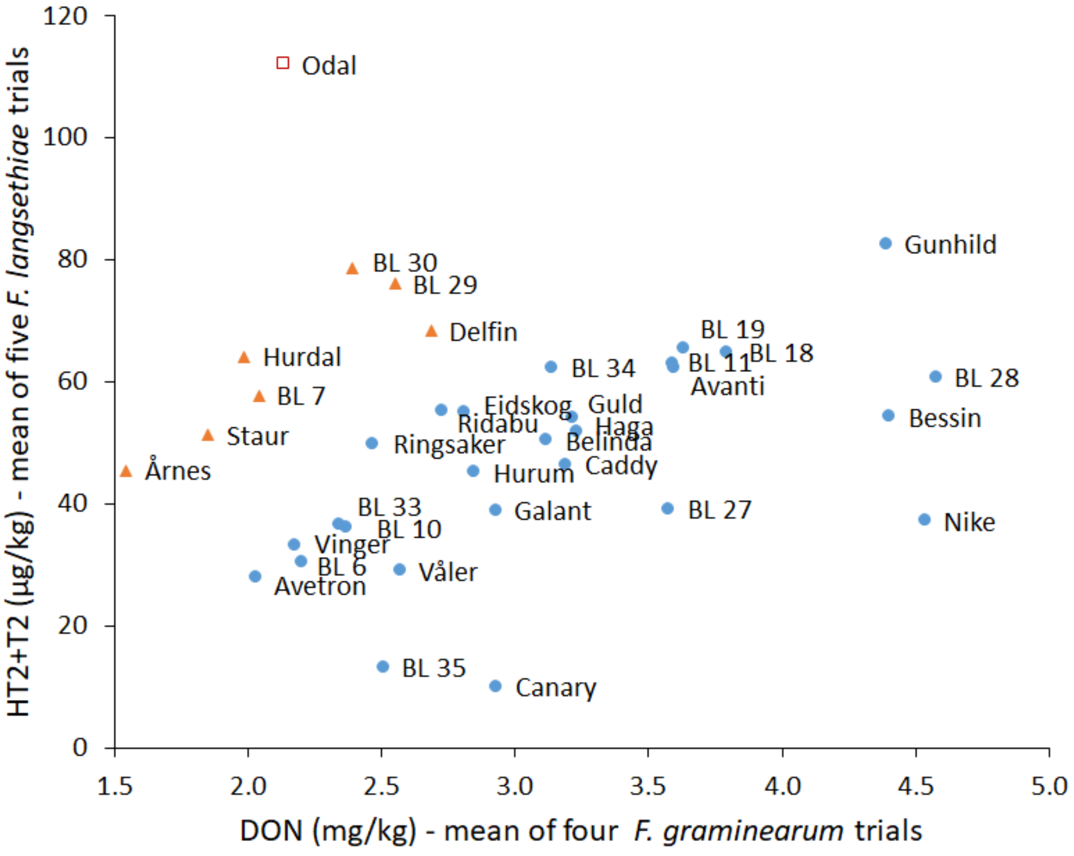

**Figure 1** The relationship between average levels of HT2+T2 in grains from oat cultivars grown in *F. langsethiae* inoculated trials versus average DON levels in *F. graminearum* inoculated trails. Released cultivars are shown with their proper names. BL = breeding line. Lines with higher susceptibility to *F. langsethiae* than to *F. graminerarum*, i.e. with higher HT2+T2 levels than expected based on the DON data are indicated with orange triangles and the cultivar 'Odal' with a red open square.

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72. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
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# Rigorous phenotypic selection for Fusarium head blight resistance yields winter wheat lines combining superior FHB resistance with good agronomic traits

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## Abstract

Breeding improved winter wheat cultivars means to select lines with combinations of a multitude of relevant traits, particularly grain yield and yield stability, end-use quality and resistance to the relevant biotic and abiotic stresses. More than 30 traits need to be considered simultaneously, making wheat one of the most complex crops to breed. Multi-trait selection is a difficult topic. Line breeding typically selects highly heritable traits in early generations often relying on visual scores and the more difficult traits that require replicated and more costly phenotyping in later stages of line selection. Selection for one trait may lead to wanted or unwanted correlated selection responses in other traits. Even for non-correlated traits selection for some traits in early generations of a breeding population may reduce the available variation for other traits in later selection rounds. These factors may be reasons why even in populations descending from crosses of resistant parents, not the best resistant progeny will be selected, particularly if the resistance trait will be evaluated later in the breeding scheme and only a small number of lines remain available for final evaluation and advancement as cultivar candidates. In this selection experiment we wanted to answer two questions: (1) Does phenotypic selection for high Fusarium head blight (FHB) resistance in early generations of a line selection scheme lead to a significant selection response towards improved resistance?; (2) Does selection for high FHB resistance in early generations of a line breeding scheme lead to correlated selection responses in other traits, such as plant height, earliness or grain yield?

The populations under investigation descend from five crosses of own highly resistant winter wheat breeding lines (Sumai 3/Capo//Hermann or Sumai 3/Capo//Toras) with the moderately resistant cultivars Midas or Amicus. Starting with 2888 $F_{2:3}$ head rows these underwent phenotypic selection through three selection rounds under artificial *Fusarium culmorum* inoculation. In each selection cycle resistant lines with low average FHB severity, not too tall plant height, healthy leaves and generally ’good-looking’ plant types have been chosen. Finally, 240 $F_{4:5}$ lines were bulk harvested and seed multiplied. From these 231 selected $F_4$-derived lines together with their parents and several control cultivars were tested during two seasons for FHB response in *F. culmorum* inoculated field trials and for grain yield in 6m$^2$ yield plots with two replications per season and employing standard agronomic practice. In a nutshell: 120 kg ha$^{-1}$ N fertilizer spit in two applications, one herbicide application in spring for weed control, no fungicide application.

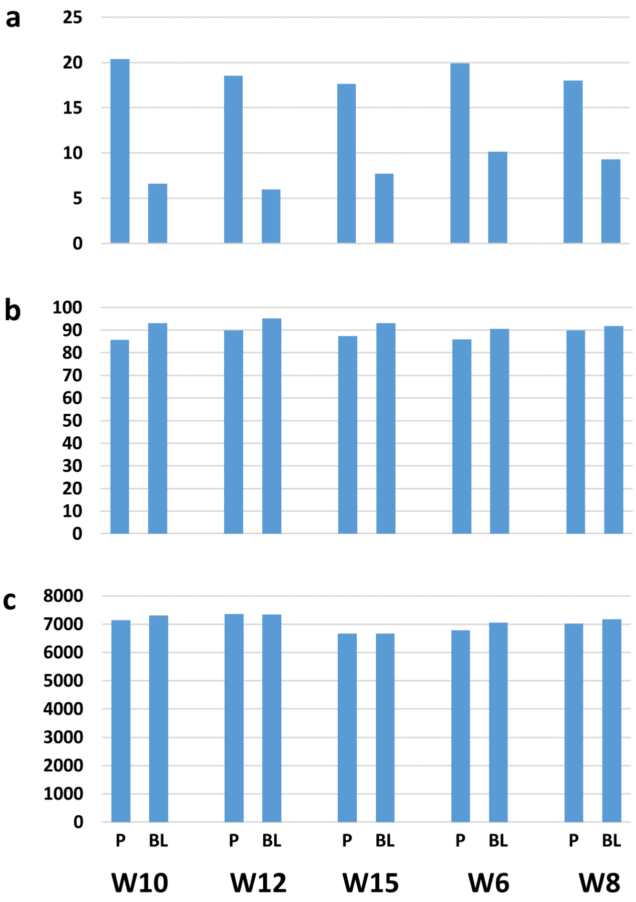

**Figure 1** Phenotypic performance of $F_4$ derived winter wheat lines (BL) in comparison to their respective parental means (P) in five families (*i.e.*, W6, W8, W10, W12, W15): **a** Average FHB severity (%) under artificial inoculation; **b** Average plant height (cm); **c** Average grain yield (kg ha$^{-1}$).

Buerstmayr H (2022) Rigorous phenotypic selection for Fusarium head blight resistance yields winter wheat lines combining superior FHB resistance with good agronomic traits. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 72. Jahrestagung 2021, 22-24 November, Raumberg-Gumpenstein, pp 15-16. University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-96-1

Selection for low FHB severity lead to a high selection response for increased FHB resistance in all five crosses tested. On average over five crosses, the selected lines displayed 57% lower FHB severity than the parental mean (Fig. 1a). The selected lines were on average 5 cm taller than their respective parental mean (Fig. 1b). Average grain yield (Fig. 1c) and average day of heading of the FHB selected lines were the same as their respective parental mean. In summary, phenotypic selection for low FHB symptoms during three years shifted the population mean significantly towards higher FHB resistance and taller plants, but did not shift the population mean for yield or earliness. Among the top 22 lines for grain yield, 10 surpassed the recently released cultivar Aurelius and 20 the cultivar Midas in grain yield. The results indicate that: (1) Selection for FHB resistance is effective in early generations of line breeding when artificial inoculation is used carefully; (2) Selection for FHB resistance does not impact grain yield. In other words: among Fusarium resistant lines, highly productive ones can be found; (3) Selection for high FHB resistance tends to favor taller breeding lines.

### Keywords

Grain yield · plant height · selection gain · selection response · *Triticum aestivum*

### Seed availability

Seeds of the breeding lines from this study can be obtained from the author upon request and will be associated with an MTA. Lines will be available for any breeder or researcher for crossing and further breeding or further research without restrictions. In case any of the lines themselves will be further developed and released as commercial cultivar, a license agreement has to be negotiated with BOKU.

### Acknowledgments

Many thanks to Sebastian Michel for performing the skillful statistical analysis of the field data.

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22.-24. November 2021, Online Conference, Raumberg-Gumpenstein, Österreich

# Genomic prediction of Fusarium head blight resistance in the WheatSustain winter wheat training set

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## Abstract

Fusarium head blight (FHB) is an economically important disease of wheat (*Triticum aestivum*). Resistance to FHB is quantitatively controlled, has significant genotype by environment interactions, and is correlated with phenological traits, such as anthesis date, plant height, and anther retention. We propose to improve genomic prediction for FHB resistance by utilizing validated loci, leveraging correlations with high-heritability traits, and incorporating weather data. Here we present preliminary findings from the SusCrop – ERA-NET project "WheatSustain: Knowledge-driven genomic predictions for sustainable disease resistance in wheat".

The WheatSustain winter wheat training set comprises 230 breeding lines from the Bavarian State Research Center for Agriculture (LfL) (*n* = 32, Germany), Graminor/Norwegian University of Life Sciences (*n* = 30, Norway), Saatzucht Breun (*n* = 59, Germany), Saatzucht Donau (*n* = 50, Austria), and Secobra Saatzucht (*n* = 59, Germany). It was genotyped with a 25K single nucleotide polymorphism (SNP) chip (SGS-TraitGenetics GmbH, Gatersleben). The training set was screened for FHB severity in Feldkirchen, Germany (Secobra), Reichersberg, Austria (Saatzucht Donau), Tulln, Austria (BOKU), and Vollebekk, Norway (NMBU) in 2020 and 2021. Trials were artificially inoculated with *Fusarium culmorum* or *F. graminearum* and mist irrigated during the anthesis/inoculation period to ensure high disease pressure. All plots were evaluated for FHB severity (%), anthesis date, anther retention, and plant height. Weather stations at each trial location collected hourly data on precipitation and temperature from sowing to harvest.

A principal component analysis of the SNP data demonstrated that the first two principal components (PCs) explained 30% of the variance in the training set, with PC1 (20% explained variance) showing separation between the Saatzucht Donau lines and the other material, and PC2 (10%) showing some separation between the Norwegian lines and the German and Austrian lines. FHB severity was positively correlated with anthesis date ($r = 0.83$) and anther retention ($r = 0.62$) and negatively correlated with plant height ($r = -0.21$). Anther retention was positively correlated with anthesis date ($r = 0.48$) and negatively correlated with plant height ($r = -0.22$). Broad-sense heritability ($H^2$) was high for anthesis date ($H^2 = 0.93$) and plant height ($H^2 = 0.9$) and moderately high for FHB ($H^2 = 0.72$) and anther retention ($H^2 = 0.73$). Genome-wide association (GWA), with control of population structure using a kinship matrix derived from the 25K SNPs, identified two SNPs significantly associated with FHB severity in the training set on chromosome 4D at 18.78 Mbp and 25.99 Mbp. The significant GWA SNPs each explained a small proportion of the variance in FHB severity ($R^2 = 0.04$, effect = 1%) and were in high linkage disequilibrium with each other ($R^2 = 0.65$). The SNP at 18.78 Mbp is a marker for the semi-dwarfing allele *Rht-D1* and was also significantly associated in GWA for plant height, where the allele conferring reduced plant height also conferred increased FHB severity. The two *Rht-D1* alleles were present in approximately equal proportions in the German and Norwegian material but only the non-semi-dwarf allele was found in the Austrian material. For genomic prediction of FHB severity within the training set, we used cross-validated

Morales L, Akdemir D, Girard AL, Holzapfel J, Sánchez JI, Kempf H, Lillemo M, Löschenberger F, Michel S, Nannuru VKR, Stadlmeier M, Steiner B, Buerstmayr H (2022) Genomic prediction of Fusarium head blight resistance in the WheatSustain winter wheat training set. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 72. Jahrestagung 2021, 22-24 November, Raumberg-Gumpenstein, pp 17-18. University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-96-1

(5-fold, 10 replicates) genomic best linear unbiased prediction (GBLUP). GBLUP is a mixed linear model in which the phenotypic response (FHB) is regressed against a random genotypic effect with variance modeled as the kinship matrix. We also tested the use of the *Rht-D1* SNP and phenological traits (anthesis date, anther retention, plant height) as fixed covariates in GBLUP. Of the eight models tested, standard GBLUP had the lowest predictive ability (PA) for FHB (PA = 0.77 ± 0.06), followed by GBLUP+*Rht-D1* (PA = 0.80 ± 0.05) and GBLUP + Height (PA = 0.80 ± 0.06). PA was highest with GBLUP + AnthesisDate (PA = 0.85 ± 0.05) and GBLUP + AnthesisDate + *Rht-D1* (PA = 0.87 ± 0.03), followed by GBLUP + AntherRetention + *Rht-D1* (PA = 0.84 ± 0.04) and GBLUP + AntherRetention (PA = 0.81 ± 0.05) (Fig. 1). These results demonstrate that incorporating FHB-correlated phenological traits and SNPs identified via GWA can improve predictive ability of GBLUP for FHB resistance in the WheatSustain winter wheat training set. We plan to do further testing on genomic prediction for FHB severity, including multi-trait and weather modeling.

## Keywords

GBLUP · genome wide assocation · phenological covariates · *Rht-D1* · *Triticum aestivum*

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This work was funded by the Austrian Federal Ministry of Agriculture, Regions and Tourism (BMLRT; grant number 101402), the Research Council of Norway (grant number 299615), and the German Federal Ministry of Education and Research (grant number 031B0810) within the ERA-NET Cofund on Sustainable Crop Production (grant number 771134) of the Joint Programming Initiative on Agriculture, Food Security and Climate Change.

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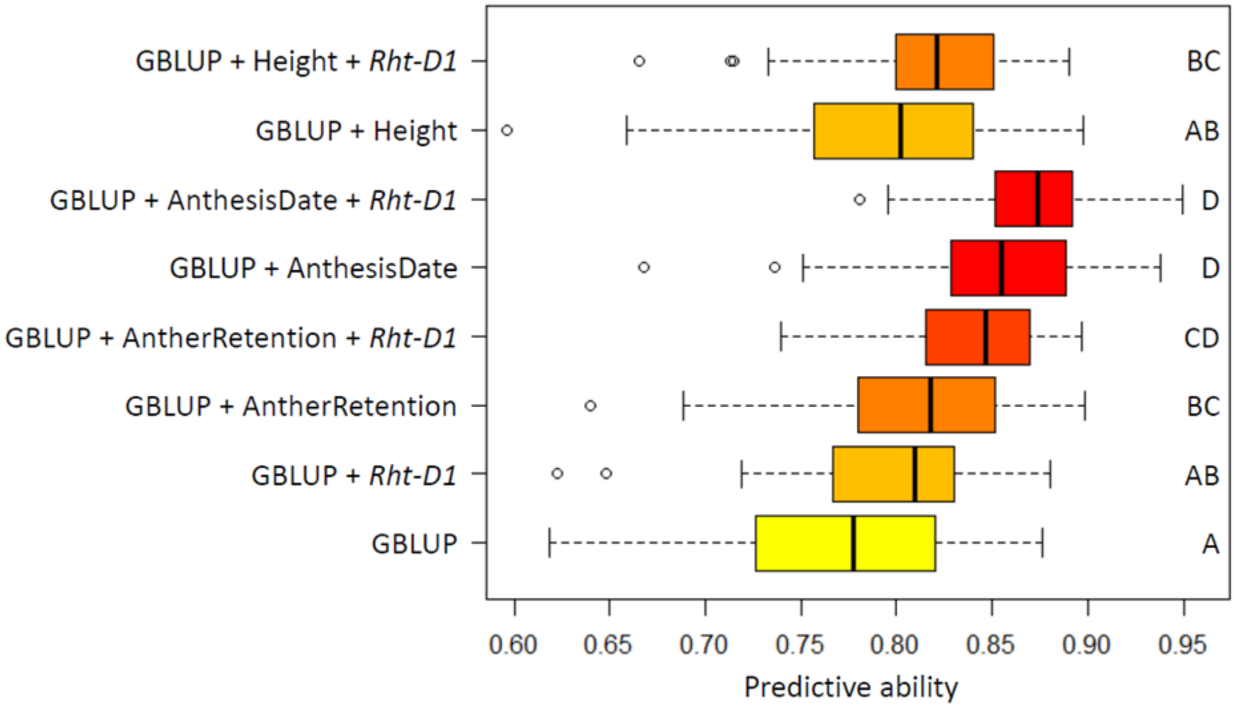

**Figure 1** Predictive ability for Fusarium head blight severity of genomic best linear unbiased prediction (GBLUP) models including the *Rht-D1* marker and phenological traits as covariates. Each model was five-fold cross-validated with 10 replications. Predictive ability refers to the Pearson's correlation between the observed and predicted values of the test set within each fold of each replication. Models that are not connected by the same letter have significantly different predictive ability (Tukey's HSD test, $p < 0.05$).

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# Merging genomics and transcriptomics for predicting Fusarium head blight resistance in wheat

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## Abstract

Genomic selection with genome-wide distributed molecular markers has evolved into a well-implemented tool in many breeding programs during the last decade. The resistance against Fusarium head blight (FHB) in wheat is probably one of the most thoroughly studied systems within this framework. Aside from the genome, other biological strata like the transcriptome have likewise shown some potential in predictive breeding strategies but have not yet been investigated for the FHB-wheat pathosystem. The aims of this study were thus to compare the potential of genomic with transcriptomic prediction, and to assess the merit of blending incomplete transcriptomic with complete genomic data by the single-step method. A substantial advantage of gene expression data over molecular markers has been observed for the prediction of FHB resistance in the studied diversity panel of breeding lines and released cultivars. An increase in prediction ability was likewise found for the single-step predictions, although this can mostly be attributed to an increased accuracy among the RNA-sequenced genotypes. The usage of transcriptomics can thus be seen as a complement to already established predictive breeding pipelines with pedigree and genomic data, particularly when more cost-efficient multiplexing techniques for RNA-sequencing will become more accessible in the future.

## Keywords

Disease resistance · genomic prediction · omics-based prediction · RNA-sequencing · *Triticum aestivum*

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# Phenotypic and genotypic analysis of a European winter wheat panel for resistance against Fusarium head blight

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## Abstract

Fusarium head blight (FHB) is one of the major diseases threatening wheat production around the world. FHB not only decreases yield, but it also produces mycotoxins which are toxic for humans and animals. Wheat production and particularly wheat quality needs to be secured. At the moment, the most common approaches to combat FHB are based on fungicides and crop management but those techniques are not sufficient to avoid damage under epidemic situations. Breeding wheat lines which are resistant to FHB, in an integrated management strategy, is the most efficient and sustainable way to control the disease.

We used the winter wheat training set (230 breeding lines from Germany, Norway, and Austria) from the SusCrop ERA-NET "WheatSustain" project to identify regionally adapted and resistant material and to assess and characterize the genetic architecture of resistance to FHB. The training set was planted at IFA-Tulln in 2020 and 2021 with a randomized complete block design with two replications per year. Overall, 920 plots were artificially inoculated with *Fusarium culmorum* and visually assessed for severity of FHB symptoms, anthesis date (AD), anther retention (AR) and plant height (PH). We used phenotypic data to investigate trait correlations and heritability. The phenotypic data was combined with genotypic information on 25K single nucleotide polymorphisms (SNPs) in a genome wide association study (GWAS) to identify quantitative trait locus (QTL) associated with resistance to FHB in this European wheat gene pool. These results may be used to improve wheat resistance to FHB through the introgression of resistance QTL or indirect selection of FHB-associated traits.

The results showed that AD, AR and PH were significantly correlated with FHB symptoms and that all traits were highly heritable ($h^2$ = 0.6-0.9). Taller lines with an early anthesis date and a lower anther retention level were associated with higher resistance to FHB (Fig. 1). In agreement with other studies, we found that a low level of retained anthers was correlated with higher resistance against FHB ($r$ = -0.62***). Retained anthers are trapped within the wheat floret and decay in it, a phenomenon which favors FHB development, while fully extruded anthers have no disease enhancing effect. As AR is a highly heritable trait ($h^2$ = 0.6) and does not negatively impact agronomic performance, it can be used as an indirect selection criterion to improve FHB morphological resistance. PH was positively but weakly correlated with FHB resistance ($r$ = 0.22***) and anther retention ($r$ = -0.19**). Additionally, GWAS revealed that the semi-dwarfing allele *Rht-D1b* was associated with higher susceptibility to FHB. Semi-dwarfing genes are frequently introduced in wheat to decrease plant height and thus lower the risk of lodging, but this also indirectly decreases resistance to FHB. The scoring of AD, AR and PH can also be used to correct FHB scores and improve physiological resistance to FHB.

## Keywords

Disease resistance · genome wide association study · trait correlation · *Triticum aestivum*

## Acknowledgements

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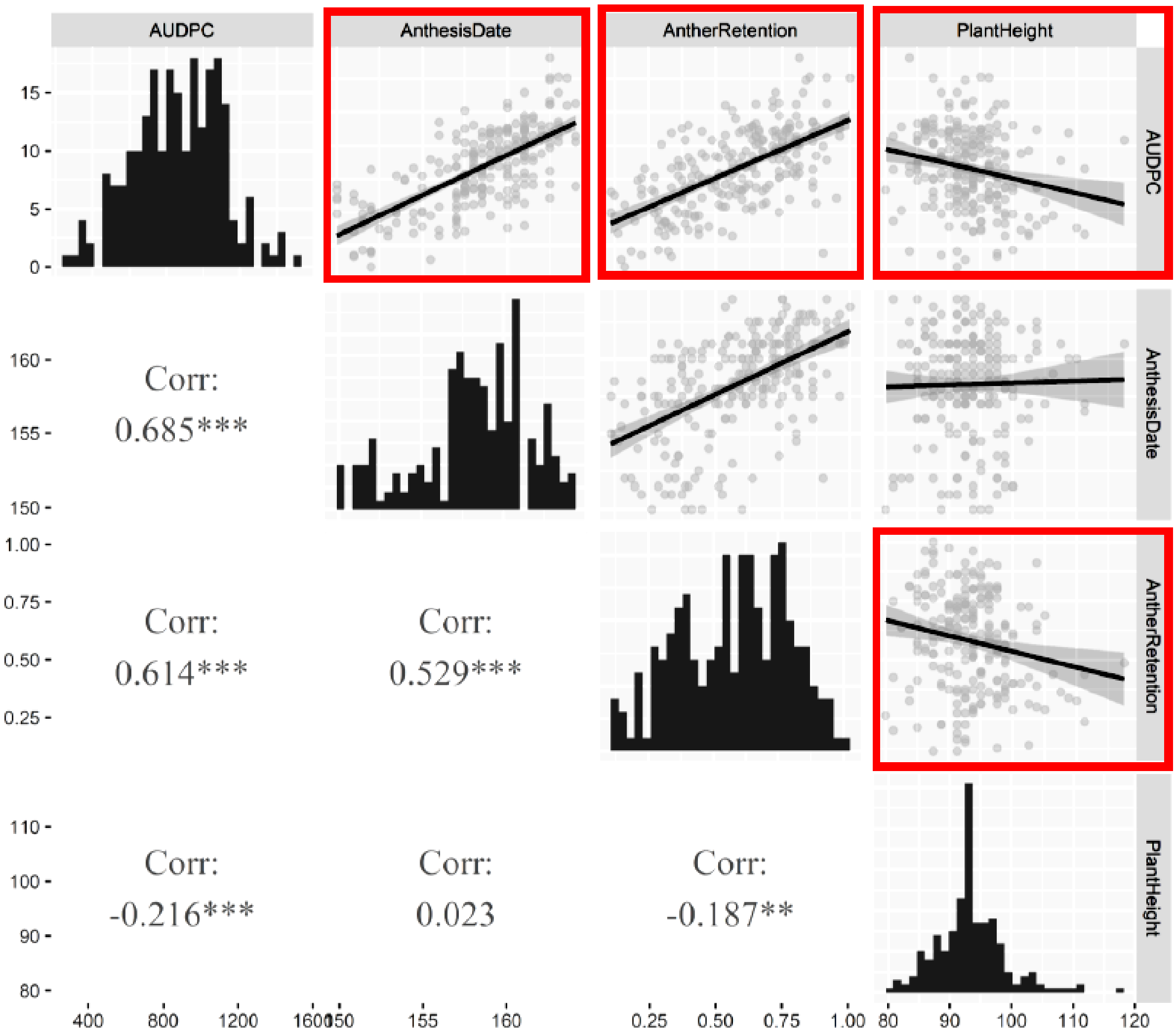

**Figure 1** Scatter plot matrix of genotypic correlations (based on genotype best linear unbiased predictors for each trait) of traits assessed in 2020 and 2021. Significance levels: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

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# Austrian wheat varieties: Influence of plant height and dwarfing genes on susceptibility to Fusarium head blight

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## Abstract

In wheat breeding, the dwarfing genes *Rht-B1* and *Rht-D1* are often critically important. However, these genes are associated with a higher susceptibility to Fusarium head blight (FHB) and toxin load. Out of 51 currently or formerly registered winter wheat varieties in Austria, two contain the dwarfing allele *Rht-B1b* and six contain *Rht-D1b*. The two alleles are believed to be less frequent in varieties registered in Austria compared to the German, French, British or Danish list of wheat varieties. A significant negative correlation was found between plant height and susceptibility to FHB ($r$ = -0.36** to -0.65**). This makes it more difficult to breed semi-dwarf wheat varieties with an above-average FHB resistance, but does not entirely rule it out. The 79 winter wheat varieties currently registered in Austria reveal low to high susceptibility to FHB. Despite intensive research and investment of many years, significant breeding progress in overall FHB resistance is still lacking.

## Keywords

Disease resistance · *Rht* genes · semi-dwarf wheat · *Triticum aestivum*

## Introduction

In recent decades, one important breeding goal in wheat (*Triticum aestivum* L.) has been to reduce plant height. This goal was pursued in tandem with attempts to reduce lodging, to improve the response to nitrogen fertilisation, to achieve an increased harvest index and to increase the grain yield (Griffiths *et al.,* 2012). However, some dwarfing genes are associated with a higher susceptibility to Fusarium head blight (FHB). The disease is mainly caused by *Fusarium graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae*. In addition to the damage to yield and quality caused by FHB, the contamination of grains with toxins such as deoxynivalenol (DON) and zearalenone (ZEA) is a particular problem. Because even small amounts of Fusarium toxins are harmful, the EU Commission defined maximum levels for these toxins in a regulation (No 1881/2006).

## Material and methods

### *Field trials & plant material*

This study is based on results of Austrian VCU tests conducted with winter wheat from 1985 to 2021. In the trials, the varieties were grown 2 to 6 times. To assess plant height, three trial series (*i.e*. Pannonian region with 79 varieties, Alpine foothills and Styria with 99 varieties, and organic trials with 20 varieties) were evaluated separately. Genotypes tested in several trial series were assigned to the most relevant respective series. A growth regulator was not used in most of the trials.

### *Traits*

Plant height was measured with the heads erect, excluding awns. Incidence and severity of FHB were assessed on a scale from 1 to 9 by the visual estimation of bleached spikelets (1 = no infestation, 9 = severe infestation). This scale is derived from a percentage scale (BAES, 2015). Symptoms were usually assessed once or twice, sometimes also three or four times. Data was mainly collected from natural or semi-natural infections. Since 2005/06, the maize stubble method has been performed annually at one or two locations. The application of maize stubbles increases the likelihood of natural infection, but avoids the technical disadvantages of minimum tillage. The likelihood of infection is further increased if the wheat plant is moist at flowering. The information about the presence or absence of the alleles *Rht-B1b* and *Rht-D1b* was obtained from Knopf *et al.* (2008) and Würschum *et al.* (2017).

### *Statistical analysis*

Due to the long time span, no orthogonal data sets are available. Adjusted means and Pearson correlation coefficients were calculated using IBM SPSS Statistics 26.

## Results and discussion

The height of a wheat plant results from the number and length of the internodes and the spike. Wheat grown from the 1920s to the 1950s often reached a height of 130 cm to 150 cm. For more than seven decades, reducing the growth and consequently the height of wheat has been a common breeding goal. Semi-dwarf varieties with improved lodging tolerance, together with higher nitrogen

Oberforster M (2022) Austrian wheat varieties: Influence of plant height and dwarfing genes on susceptibility to Fusarium head blight. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 72. Jahrestagung 2021, 22-24 November, Raumberg-Gumpenstein, pp 23-26. University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-96-1

fertilisation, has contributed significantly to increasing wheat yields. However, lodging is influenced by many factors other than plant height. In some enviornments, reduced plant height can result cause yield loss, *e.g*. drought and heat often damage short-strawed wheat varieties more than tall varieties.

The Japanese genotype 'Norin 10' is considered to be the source of *Rht-B1b* (formerly *Rht1*) and *Rht-D1b* (formerly *Rht2*), which have been widely introduced into wheat varieties since the 1960s. The *Rht1* alleles are located on chromosomes 4BS and 4DS (Börner *et al.,* 1996), and they restrict the response to the growth-promoting hormone gibberellin. In total, 19 *Rht* genes have been described in wheat. Seven are mutated alleles of *Rht1* and several are alleles of *Rht8* (Sukhikh *et al.,* 2021). Only a few of these are used in practical breeding. Among German winter wheat varieties, 36-38% and 4-6% had the allele *Rht-B1b* and *Rht-D1b*, respectively (Knopf *et al.,* 2008; Würschum *et al*., 2017).

Among the 51 winter wheat varieties currently or formerly registered in Austria, two ('Renan', 'Tulsa') contain *Rht-B1b*, and six ('Chevalier', 'Contra', 'Dekan', 'Jenga', 'Tambor', 'Toras') contain *Rht-D1b*. The two gene variants are also believed to be less frequent in varieties registered in Austria compared to wheat varieties registered in Germany, France, Great Britain, or Denmark. In any case, these variants are less frequent in wheat varieties that are mainly cultivated in the Pannonian region and on organic farms.

On the other hand, many of the wheat varieties used in Austria's humid region are believed to be carriers of *Rht-B1b* or *Rht-D1b*. These varieties largely originate from breeding programmes run by companies in Germany, France and the Netherlands. However, the plant height of wheat varieties is not only the result of the few *Rht* genes, but is also determined by numerous quantitative trait loci (QTL) which have medium or low effects (Griffiths *et al.,* 2012). The plant heights of the investigated varieties varied from 73 cm for 'Victo' to 115 cm for 'Erla Kolben' (Pannonian region series) and from 77 cm for 'Tulsa' to 115 cm for 'Rektor' (humid region series). The wheat varieties grown on organic farms displayed plant heights ranging from 89 cm ('Bitop', 'Blasius', 'Exklusiv') to 108 cm ('Arminius'). The fact that the tallest varieties do not appear in the organic series can be explained by the later start of the organic VCU trials in 2001 with the first varieties released three years later. The negative effect of *Rht-B1b* and *Rht-D1b* on FHB resistance has been clearly documented (Draeger *et al*., 2007; Miedaner & Voss, 2008; Srinivasachary *et al*., 2009). This effect is also associated with anther retention/extrusion during flowering (Buerstmayr & Buerstmayr, 2016). The presence of *Rht-D1b* makes the plant more susceptible to FHB compared to *Rht-B1b* (Buerstmayr & Buerstmayr, 2016; Miedaner & Voss, 2008; Srinivasachary *et al*., 2009). This aspect is not reflected in this study due to the small number of varieties included (Table 1).

In the series of three trials (Fig. 1), plant height was negatively correlated with FHB susceptibility (*r* = -0.36** to -0.65**). This susceptibility does not present a fundamental obstacle to breeding varieties with shorter or quite short plant heights and average to above-average resistance to FHB. Examples include 'Activus', 'Advokat', 'Artimus', 'Fidelius', 'Findus', 'Monaco', 'Spontan', 'SU Habanero' and 'SU Mangold'. Some varieties that contain *Rht-B1b* or *Rht-D1b* are even only slightly or slightly to moderately susceptible to FHB (*e.g*. 'Renan', 'Chevalier', 'Toras'). For these reasons, it does not seem advisable to abandon the use of *Rht* alleles. A few years ago, *Rht24b* was detected on chromosome 6A. Unlike *Rht-B1b* and *Rht-D1b*, this dwarfing allele does not increase susceptibility to FHB (Herter *et al.,* 2018; Würschum *et al.,* 2017). It is present in many wheat varieties worldwide and is also likely to be significant in the varieties cultivated in this country.

**Table 1** Plant height and FHB score in winter wheat varieties with and without dwarfing genes (adjusted means; trials 1985-2021).

| VCU series / trait | *Rht-B1* | *Rht-D1* | No |
|---|---|---|---|
| Pannonian region (*n*) | 1 | 1 | 16 |
| Plant height (cm) | 83.5 | 89.2 | 99.6 |
| FHB (1-9) | 2.76 | 2.52 | 2.83 |
| Humide region (*n*) | 1 | 5 | 27 |
| Plant height (cm) | 76.9 | 93.0 | 104.0 |
| FHB (1-9) | 4.03 | 3.52 | 3.33 |

Among the 79 described winter wheat varieties in Austria (AGES 2022), 14 have low levels of susceptibility to FHB (*e.g*. 'Albertus', 'Arminius', 'Edelmann', 'Mandarin', 'Tilliko' and 'Tobias'). Thirty varieties are rated as having low to medium levels of susceptibility (score 4), 14 varieties are considered to have medium levels of susceptibility (score 5), 18 varieties have medium to high levels of susceptibility (score 6) and 3 varieties ('Christoph', 'Frenetic', 'WPB Calgary') have high levels of susceptibility. The varieties intended for organic farming tend to be less frequently infected by FHB than those used in conventional farming. Since the 1980s, great importance has been given to breed wheat varieties resistance to FHB, and intensive research has been carried out to do so. Nevertheless, the results of the official VCU testing (Fig. 2) do not reveal a trend towards reduced susceptibility in the Austrian winter wheat varieties, in contrast to the trend observed for resistance to powdery mildew and leaf rust.

In order to reduce costs, to protect the soil from run-off and to consider water storage issues, minimum tillage systems are now being widely used in crop production. Incompletely decomposed crop residue from maize or other cereal grains on the soil surface, however, provides an ideal nutrient substrate for Fusarium fungi. For this reason, the incidence of infection by these pathogens has increased. In addition, the fungicides currently used against FHB are only effective to a limited extent. A Farm to Fork strategy has been developed by the EU Commission to significantly reduce the use of pesticides. Furthermore, considerations are being made to reduce the limit values for Fusarium toxins in the harvested grains of wheat and other cereals. Achieving a pronounced varietal resistance to FHB, therefore, would be both necessary and helpful.

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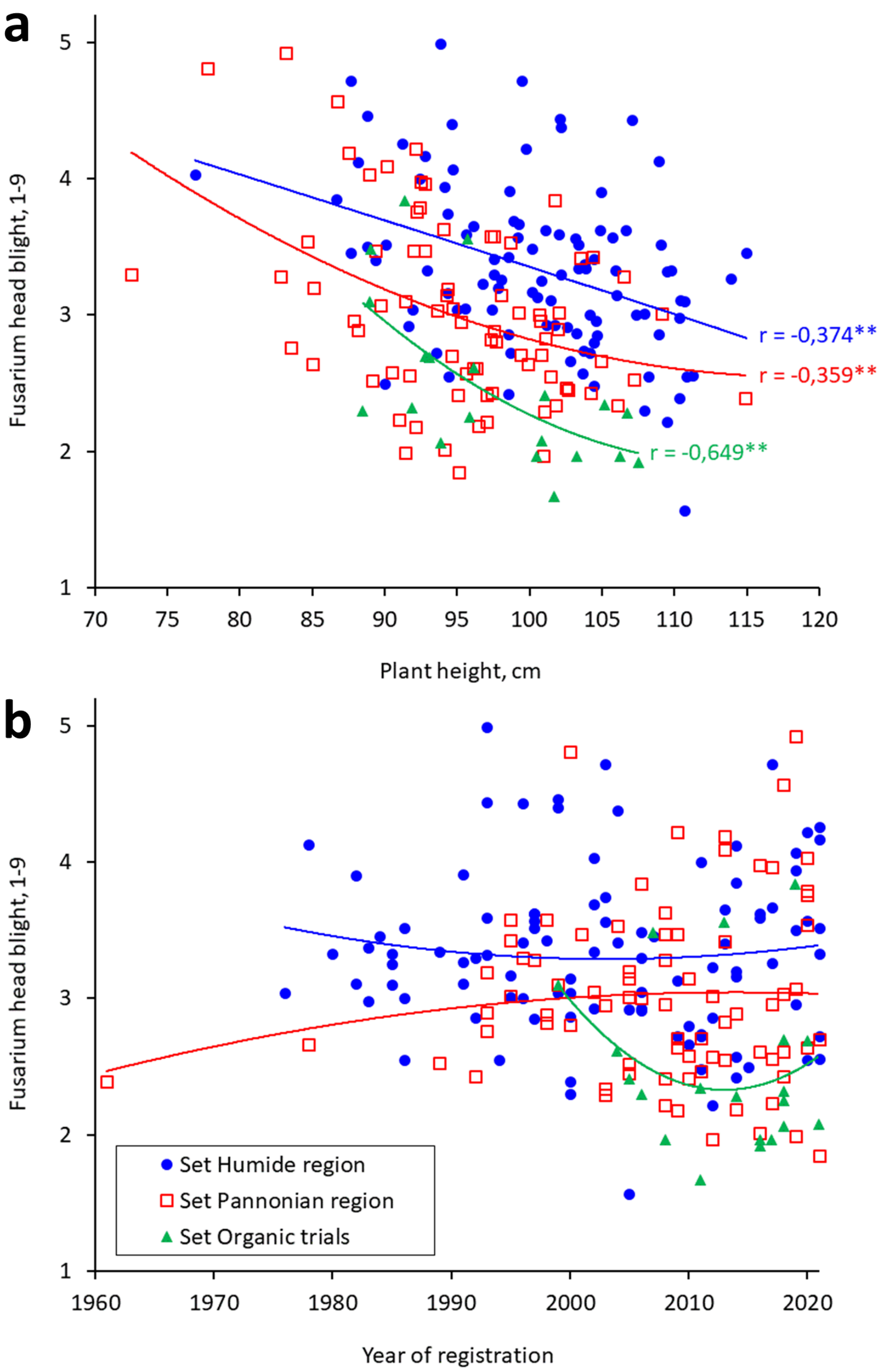

**Figure 1** FHB susceptibility of winter wheat varieties in three series of Austrian VCU trials (1985-2021) in relation to **a** plant height, and **b** year of variety release

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72. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
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# Genetic architecture of Fusarium head blight disease resistance and associated traits in Nordic spring wheat

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## Abstract

Fusarium head blight (FHB) is a devastating fungal disease affecting the wheat production worldwide caused by *Fusarium graminearum* and other *Fusarium* species. FHB resistance is quantitative, highly complex and divided into several resistance types. Mycotoxins, such as deoxynivalenol (DON) which are produced by *F. graminearum* may cause severe problems and is a threat to both animals and humans, reaching from feed refusal and poor weight gain in animals to immunological problems in humans. Breeding for disease resistance is the most cost-effective method to control this disease. To develop resistant cultivars, proper understanding of resistance types or mechanisms is required. Quantitative trait loci (QTL) that are effective against several of the resistance types would thus be a valuable contribution for resistance breeding.

New marker technologies, enabling QTL detection, association mapping and subsequently marker detection, have the potential to increase the efficacy of resistance breeding for FHB and in addition dissect and enhance the understanding of the genetic basis of the complex resistance mechanisms. In recent years, genome-wide association studies (GWAS) for detection of FHB resistance QTL have been conducted. The benefit of these studies is the ability to capture historic recombination events and utilize collections with a wide genetic background. This increases the possibility for breeders to detect interesting QTL for resistance directly in breeding relevant germplasm and enable a targeted incorporation of resistance QTL into their breeding programs. QTL for both FHB disease severity and DON content can serve as valuable sources for disease resistance breeding in wheat. SNP markers closely linked to the resistance QTL could be further tested and used in resistance-breeding for FHB and DON resistance.

The NMBU spring wheat panel of 300 cultivars and breeding lines with different geographical origins was tested in artificially inoculated field trials and subjected to visual FHB assessment in the years 2013 to 2015, 2019 and 2020. Principal component analysis based on 35K and 25K SNP arrays revealed the existence of two subgroups among the cultivars and breeding lines of NMBU spring wheat panel, dividing the panel into European and exotic lines. DON content was measured on harvested grain samples and anther extrusion (AE) was assessed in separate trials. In addition to the above assessments, plant height and days to heading were also assessed in all the field trails.

GWAS was conducted to detect QTL associated with FHB traits and identify marker-trait associations (MTAs) that consistently influenced FHB resistance. Various GWAS models were tested, from which FarmCPU was chosen for its proven efficiency over other models in several recent studies. We detected 13 significant QTL regions in this GWAS for the FHB resistance (Table 1). Significant markers from these QTL regions were tested for their effect on FHB disease resistance with the help of haplotype and allele stacking analyses. From these analyses, we were able to identify the resistant haplotypes and the lines contributing the resistance based on traits assessed, such as FHB disease severity, DON content, and anther extrusion. Of importance, haplotype analysis

Nannuru VKR, Windju SS, Belova T, Dieseth JA, Alsheikh M, Dong Y, McCartney CA, Henriques MA, Buerstmayr H, Michel S, Meuwissen THE, Lillemo M (2022) Genetic architecture of Fusarium head blight disease resistance and associated traits in Nordic spring wheat. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 72. Jahrestagung 2021, 22-24 November, Raumberg-Gumpenstein, pp 27-29. University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-96-1

**Table 1** Overview of the most significant and consistent QTL detected in the present GWAS, and comparison with previously published FHB resistance QTL based on positions on the wheat reference genome assembly (RefSeq v1.0; IWGSC, 2018) .

| QTL | Physical map position (Mbp) | -log(p) value | Germplasm[1] | Published References |
|---|---|---|---|---|
| *Qfhb.nmbu.1A.1* | 9 - 46 | 3.05 - 13.27 | NMBU + Europe | Jiang *et al*. (2007a,b); Buerstmayr *et al*. (2009); Liu *et al*. (2009); Sari *et al*. (2018); Venske *et al*. (2019); Zhu *et al*. (2020) |
| *Qfhb.nmbu.1A.2* | 520 - 590 | 3.04 - 8.16 | NMBU | Venske *et al*. (2019); Zheng *et al*. (2021) |
| *Qfhb.nmbu.3A.1* | 683 - 738 | 3.09 - 12.27 | NMBU + Europe | Venske *et al*. (2019) |
| *Qfhb.nmbu.3B.1* | 7 - 9 | 3.49 - 12.86 | NMBU | Anderson *et al*. (2001); Liu *et al*. (2006); Buerstmayr *et al*. (2009) |
| *Qfhb.nmbu.4A.1* | 537 - 607 | 3.20 - 9.21 | NMBU | Zheng *et al*. (2021) |
| *Qfhb.nmbu.4A.2* | 721 - 743 | 3.09 - 8.40 | NMBU + Europe | present study |
| *Qfhb.nmbu.4B.1* | 527 - 609 | 3.02 - 6.41 | Europe | present study |
| *Qfhb.nmbu.5A.1* | 480 - 552 | 3.0 - 12.49 | NMBU + Europe | Venske *et al*. (2019); Zheng *et al*. (2021) |
| *Qfhb.nmbu.6A.1* | 450 - 531 | 3.06 - 6.66 | NMBU + Europe | present study |
| *Qfhb.nmbu.6A.2* | 608 - 609 | 3.09 - 5.73 | NMBU + Europe | Ruan *et al*. (2020) |
| *Qfhb.nmbu.6B.1* | 630 - 688 | 3.03 - 6.55 | NMBU + Europe | present study |
| *Qfhb.nmbu.7A.1* | 120 - 129 | 3.05 - 3.86 | NMBU + Europe | present study |
| *Qfhb.nmbu.7A.2* | 670 - 710 | 3.50 - 25.45 | NMBU + Europe | Semagn *et al*. (2007); Buerstmayr *et al*. (2009); Sari *et al*. (2018); Wu *et al*. (2019); Ruan *et al*. (2020) |

[1] NMBU, NMBU spring wheat panel; Europe, European panel(s)

revealed a highly significant and consisted QTL on 7A, *Qfhb.nmbu.7A.2,* which was further validated on an independent set of breeding lines. Breeder-friendly KASP markers were developed for this QTL that can be used in marker-assisted selection. The lines in the wheat panel harboured from zero to five resistance alleles, and allele stacking showed that resistance can be significantly increased by combining several of these resistance alleles.

Moreover, the resistance sources and QTL identified in this study will facilitate a further genetic improvement of FHB resistance in Nordic and European wheat germplasm by genomic breeding strategies. This information on FHB resistance and an accelerated stacking of multiple resistance alleles enables and enhances breeders´ possibilities for genomic prediction and to breed cultivars with improved FHB resistance.

**Keywords**

Deoxynivalenol (DON) · *Fusarium graminearum* · genome-wide association study · marker-trait association · quantitative trait loci · *Triticum aestivum*

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72. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
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# Genome-wide association study for resistance to stripe rust in Central European winter wheat

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## Abstract

Stripe rust or yellow rust, caused by the fungus *Puccinia striiformis* (*Pst*), is one of the most destructive diseases of wheat (*Triticum aestivum* L.). It leads to a significant economic damage with losses up to 70-80% in epidemic conditions and about 5.47 million tonnes of total grain yields annually. Sustainable management of wheat stripe rust is achievable by identifying, introducing and subsequently selecting rust resistance genes during the breeding cycles. A total of 83 stripe rust resistance genes (*Yr*) have been already identified in wheat. Virulent *Pst* races against most *Yr* single genes have been emerged so far, therefore, continuous efforts to characterize new resistance genes with tightly linked molecular markers are needed. The selection and combination of acting genes already present in the advanced breeding pool is also essential to enhance durable stripe rust resistance and for the development of new wheat cultivars.

An association mapping panel comprising 230 winter wheat cultivars and breeding lines from Central Europe (*i.e.* Austria, Germany, Norway, Sweden, Denmark, Poland, Switzerland) was established in order to search for sources of effective resistance genes and associated QTL which can be used in future breeding programs. Using whole genome scanning (Illumina iSelect 25 K Infinium SNP-genotyping array; 8900 polymorphic markers) we detected two subpopulations, a first one including 92 Austrian breeding lines and cultivars which were separated by their different agronomic characteristics from the second one including the other 138 lines. The frequency distribution of disease severity (%) evaluated in field trials (2020-2021) located in Lemgo (52°1'40.3"N, 8° 53'56.44"E) and Lenglern (51°35'9.59"N, 9°52'8.99"E) in Germany, and in Tulln (48°19’41”N, 16°03’30”E) and Reichersberg (39°54’26” N, 116°23’50”E) in Austria were skewed slightly towards the more resistant tail implying that many effective adult plant resistance (APR) or race non-specific genes could be present in this panel.

Genome-wide association study (GWAS) using pairwise kinship matrix and mixed linear model (MLM) identified 66 SNP markers located on 13 wheat chromosomes that were associated to stripe rust disease severity evaluated at the adult plant stage in different field trials. The associated markers in a single environment explained between 5 and 11% of the total phenotypic variation ($R^2$) . Although minor, these loci still could play an important role towards stable resistance when combined with major loci in breeding programs. Three hotspot QTL regions were detected on chromosomes 2BL, 4BL and 6AL that were expressed at least in two environments. These QTL based on their physical positions on the IWGSC reference sequence of bread wheat (IWGSC RefSeq v2.1) were in the vicinity of previously mapped *Yr* genes (*i.e. Yr5*, Yr*44* and *Yr63*) and reported QTL. Further studies are required to confirm whether these QTL are likely to be distinct from these genes. Importantly, the association of two loci on chromosomes 2BS and 6AL could be validated in two validation populations of 1065 and 1002 breeding lines which have been evaluated for percentage of disease severity in Lemgo, Germany, during 2020 and 2021. Also, considerable differences in the allelic effect of these loci were observed in another independent validation population of 175 LfL breeding lines evaluated in Lenglern, Germany, in 2021. The QTL found in combination with effective all-stage resistance (ASR) could be prioritized in wheat breeding programs to obtain commercially acceptable level of stripe rust resistance in future.

## Keywords

Bread wheat · disease resistance · genetic map · GWAS · *Puccinia striiformis* · yellow rust · *Triticum aestivum*

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72. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
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# A major QTL for yellow rust resistance on chromosome 6A shows increased frequency in recent Norwegian spring wheat cultivars and breeding lines

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## Abstract

Yellow rust (YR) or stripe rust, caused by *Puccinia striiformis* f. sp. *tritici,* is one of the most devastating diseases on wheat, especially in wheat growing regions with wet and cool climates. Due to the epidemic of the new YR race group *PstS10* in Europe, yellow rust has been a reoccurring disease in Norway since 2014 and many important Norwegian wheat cultivars are susceptible to the new YR races. Although a high number of race-specific YR resistance genes or quantitative trait loci (QTL) have been identified, they can easily be overcome by the rapid evolution of the pathogen population. Thus, identifying race non-specific YR resistance QTL or genes would therefore be preferable. The main objectives of this study were to (i) investigate the YR resistance in the Nordic spring wheat materials, and (ii) identify YR resistance QTL which could be further applied in marker-assisted selection (MAS) for improving the YR resistance in Norwegian wheat breeding.

Adult plant resistance to YR was assessed in a total of 13 field trials of the Nordic spring wheat association mapping panel MASBASIS conducted at seven locations across four countries (Norway, Germany, Austria, China). The MASBASIS spring wheat panel consists of 300 lines including breeding lines and the most important current and historical cultivars grown in Norway. Genotyping was done with the 25K SNP chip by TraitGenetics GmbH. Genome-wide association analyses were done by using the FarmCPU model implemented in the R package GAPIT with the significance threshold set to FDR adjusted p-value below 0.05.

Ten consistent YR resistance QTL were identified on chromosomes 1B, 2A, 3B, 5A, 5B, 6A, 6B, and 7A, respectively. Among those, one QTL on chromosome 6A was the most significant and consistent across years and locations (Fig. 1). We suppose the YR races in Europe and China are likely different. As the QTL on 6A was significantly detected in both continents across years, the causal gene of this QTL might be a race non-specific resistance gene, which can be further utilized in broader breeding programs for more sustainable YR resistance. In addition, the haplotype effect of this 6A QTL was also validated using an independent panel of Norwegian breeding lines. Moreover, to trace the origin of the resistance source, we compared the 6A haplotypes of the whole GWAS panel, and increased frequency of the resistant haplotype was found in recent Norwegian spring wheat cultivars.

## Keywords

Genome-wide association study (GWAS) · marker-assisted selection (MAS) · *Puccinia striiformis* · quantitative trait loci (QTL) · resistance breeding · *Triticum aestivum*

## Acknowledgements

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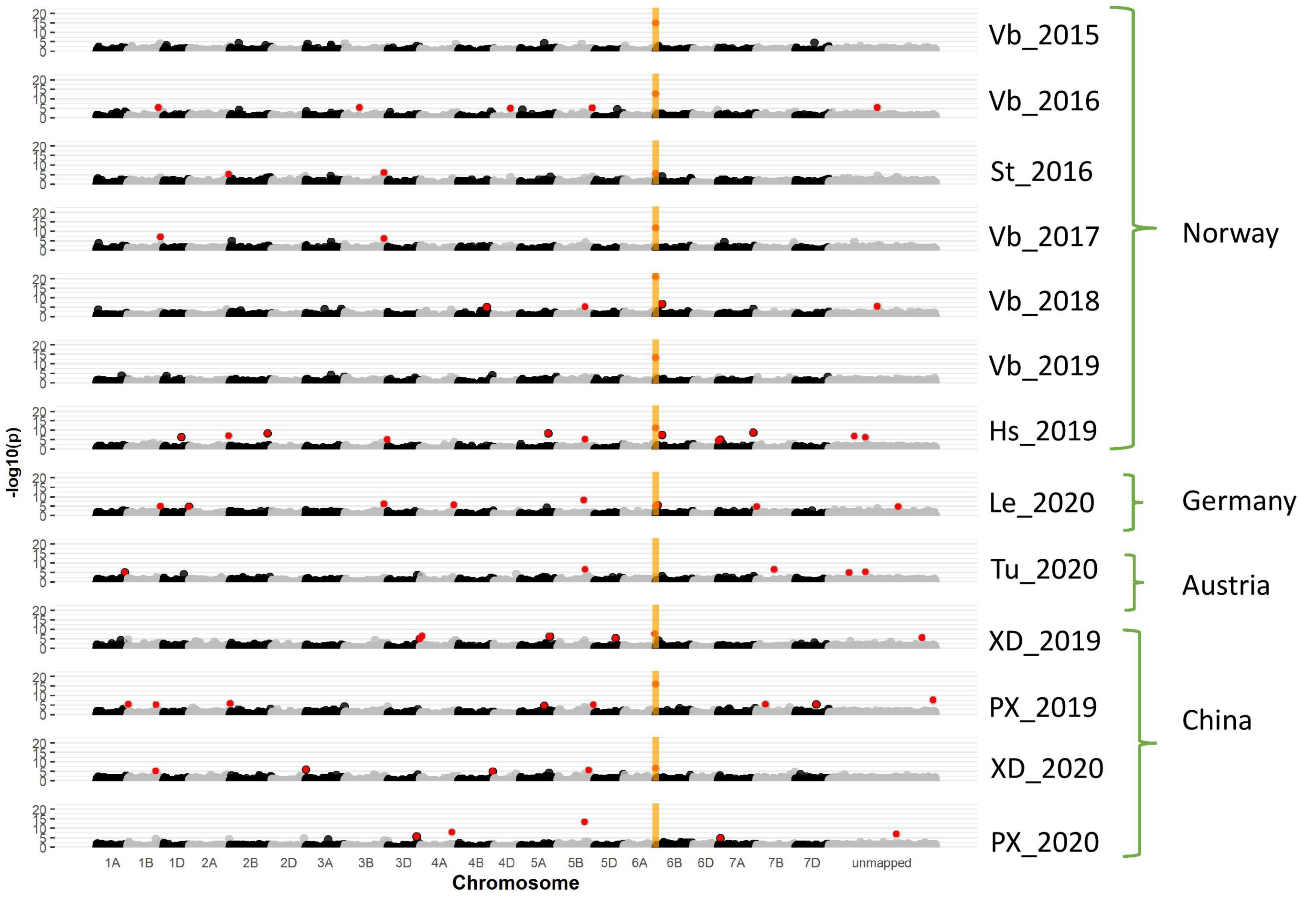

**Figure 1** Manhattan plots of genome-wide associations for yellow rust disease severity in the Nordic spring wheat panel. Vb: Vollebekk, Norway; St: Staur, Norway; Hs: Holmestrand, Norway; Le: Lemgo, Germany; Tu: Tulln, Austria; XD: Xindu, CN; PX: Pixian, China. The significance threshold set to FDR adjusted *p*-value below 0.05. Markers above the significance threshold are indicated in red dots. The region of the consis-

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# *Phs-A1* confers pre-harvest sprouting resistance independent of phenology in European winter wheat and multiple genomes reveal structural variation

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## Abstract

Pre-harvest sprouting (PHS), the germination of seeds within heads before harvest, is a major problem in global wheat (*Triticum aestivum* L.) production, manifested by the loss of bread-making quality in affected grain. Marker-assisted selection (MAS) can be a useful tool to select for PHS resistance, especially in years without pre-harvest rain. Genome-wide association studies (GWAS) are required to find markers to be used in selection, especially in a breeding context.

We tested 1000 wheat breeding lines and cultivars from across Europe in four years, *i.e.* 2017 – 2020. Lines and cultivars were genotyped for 10k genome-wide SNP markers using GBS and a commercial SNP-Array. To assess PHS in the field, we challenged lines using irrigation at maturity and subsequently measured Hagberg falling number (HFN) as well as counted the number of sprouted grains. For lab-based challenging, we cut ten heads at maturity before irrigation. These heads were soaked in water, incubated, and visually scored for sprouting progression in the following days (Fig. 1). Furthermore, we assessed phenological, agronomic, and quality-related traits to co-locate them with PHS markers. These phenotypes were used to test for association with the genome-wide marker data. From the significant associations, we selected the "strongest" across years based on the number of significant associations. To connect our results to PHS related genes from literature and possibly locate previously unassigned markers to chromosomes, we blasted markers and genes to the IWGSC RefSeq v2.1 as well as ten pan-genomes. To assess the structural variation at PHS hot-spots across the pan-genome, we aligned genomes pair-wise using MUMmer.

Hotspots of strong PHS associations were found on chromosomes 1A, 4A and 5B (Table 1). Associations on 1A and 5B are possibly novel. While markers on chromosome 5B are linked to phenology and therefore of limited use in a breeding program, associations on chromosome 1A are not linked to other traits and might therefore, be highly useful for selecting PHS resistant lines. Markers on chromosome 4A are located in an interval that is known to harbor the *Phs-A1* locus, for which two genes, *PM19* and *MKK3*, were previously reported to be causative. We found three distinct peaks with association to PHS in this region and variable arrangements of markers and genes across genomes, suggesting structural variation as a cause for inconclusive association results in this and previous studies. This result could also be caused by imperfect genome assembly since the wheat genome is notoriously hard to assemble due to it's large genome size and high content of repetitive elements. To confirm this, more long-read sequencing data and high quality assemblies are necessary, which are to date not available. However, this approach revealed *Phs-A1* as the most stable and effective locus for regulating pre-harvest sprouting independent of phenology or quality related traits in Europe and revealed that *Phs-A1* is not only influenced by allelic, but also by structural genome variation.

**Figure 1** Laboratory pre-harvest sprouting assessment on intact wheat spikes: heads were cut at maturity, soaked and incubated for 7 days; scoring on a 1 (no sprouting) to 9 (severe sprouting) scale.

**Keywords**

Genome-wide association · pan-genome · QTL · structural variants · *Triticum aestivum*

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**Table 1** Markers associated with pre-harvest sprouting (PHS) grouped by evidence strength and chromosome. Number of markers found to be significantly associated with PHS using genome-wide association per chromosome, grouped by evidence strength (weak = associated in one or two years, strong = associated in three or four years).

| evidence | 1A | 1B | 1D | 2A | 2B | 2D | 3A | 3B | 3D | 4A | 4B | 4D | 5A | 5B | 5D | 6A | 6B | 6D | 7A | 7B | 7D | Un |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| weak | 10 | 6 | 2 | 12 | 11 | 23 | 2 | 5 | 3 | 8 | 9 | 2 | 1 | 12 | 2 | 8 | 3 | 2 | 3 | 9 | 6 | 4 |
| strong | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |

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# Field phenotyping: Unmanned aerial vehicle (UAV) multispectral imaging for winter wheat survival estimation

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## Abstract

In Norway, winter survival is an important breeding goal for winter cereals and perennial forage crops due to the harsh winter climate and increasing pressure caused by the climate change. Winter in many areas often lacks protecting snow cover causing severe winterkill despite higher temperatures on average. At the same time, warmer and longer growing period promotes higher yields, putting the cultivation of winter wheat in a position of a potentially profitable but risky gamble. Manual assessments of winter survival is tedious and prone to human error due to fatigue.

This study investigates winter wheat survival estimation using UAV imagery. A multispectral camera with the 5 bands Red, Green, Blue, RedEdge, and Near Infrared (NIR) was applied to survey the whole field. Two different observers carried out the manual scoring simultaneously. Vegetation indices including Normalized Difference Vegetation Index (NDVI), and Green Normalized Difference Vegetation Index (GNDVI) were calculated. Soil pixels were masked out and the binary image was generated. Total percentage of vegetation pixels (TPVP) per plot was calculated as the ratio of vegetation pixels to the total pixels in a plot. Plots that were recorded as 100% survival based on manual scores by both observers were applied as reference for survival estimation. An image index equal to (100*TPVP)/50 was defined to estimate the survival in each plot (Fig. 1).

Winter wheat survival measurements estimated by Multispectral UAV imagery showed a strong correlation with visual assessments ($r = 0.91$ and 0.82) but outperformed them in reproducibility (broad-sense heritability $H^2 = 0.93$ vs 0.89 and 0.81) and accuracy (cv = 0.14 vs 0.17 and 0.22). UAV imagery survival estimation is time efficient in comparison to manual scoring. In conclusion the methodology is fast and straightforward and it can be applied for many crops.

## Keywords

Heritability · high-throughput phenotyping · multispectral imaging · *Triticum aestivum* · winter hardiness · winter wheat survival

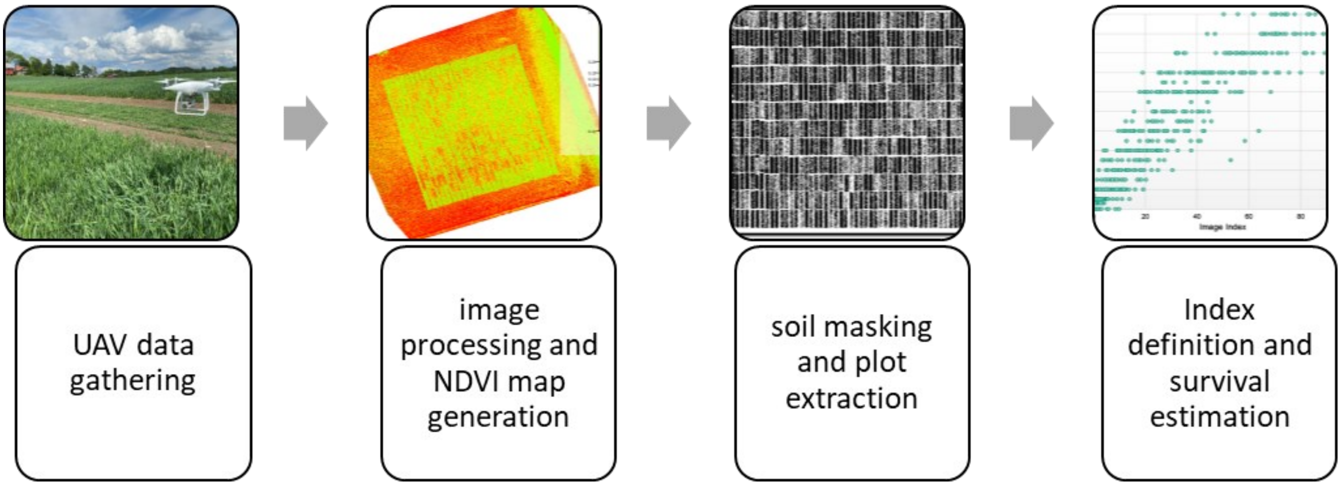

**Figure 1** Workflow of winter wheat survival estimation using unmanned aerial vehicle (UAV) imagery

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# Frost resistance of winter wheat tested within the ECOBREED project

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## Abstract

Frost resistance of winter wheat is an important traits for stability of grain yield in many countries. The required level of frost resistance differs and might be lower in Central Europe, *e.g*. the Czech Republic, compared to Russia or Canada, but cultivation of varieties without any frost resistance is very hazardous. The cold winters in the seasons 2002/2003 and 2011/2012 emphasized the importance of this trait, when more than 20% of the winter wheat acreage were completely damaged. Frost resistance is of equal importance in conventional and organic farming. In the present study, the frost survival of 80 winter wheat genotypes which are tested for their performance under organic conditions within the H2020 project ECOBREED, was tested by three methods under Czech conditions in order to evaluate the risk of winterkilling.

Many methods have been designed and are used for the artificial testing of frost resistance. They differ in the way plants are sown and prepared for testing, in the hardening phase and freezing steps and in the evaluation of frost damage. In the Czech Republic, four genotypes are used to characterize the variation in frost tolerance, *i.e*. 'Bohemia' (score 9 = excellent frost tolerance; 100% survival), 'Illusion' (7), 'Lorien' (4), and 'Aranka' (score 0 = no frost tolerance). In the current study plants were grown in seedling trays (quickpot) in peat substrate (2 mm coarseness). Hardening of young plants was done under natural conditions in November and December 2021 (methods KS and KU). For about half of the days during this period the temperature was between –5°C and +5°C. Artificial hardening in the growing chamber (method L) was done for 30 days at 5°C day and 0°C night temperature with a day length of 8 hours. Each genotype was sown in three replications with each replication submitted to a different frost stress (*i.e*. -12, -14 and -16°C). The frost treatments were applied under controlled conditions in frost chambers for 24 hours. Plant regeneration took place in a tempered greenhouse at 15-20°C. After the restoration of vegetation growth (about 14-21 days), the numbers of surviving plants were evaluated. The result of the evaluation is the survival index defined as percentage of surviving seedlings of all planted seedlings.

None of the tested genotypes reached the same high level as control variety 'Bohemia' (Fig. 1). Varieties 'Philaro', 'Liseta', 'Stupicka Bastard', 'Viki', 'Vlasta', 'Wiwa' and breeding line ST860/20 achieved very good levels of frost resistance. Varieties 'Aurelius', 'Sunnyboy', 'Genius' and 'Capo' showed a low frost resistance and their overwintering under Czech conditions could be risky. From previous studies with seeds of conventional origin, it is known that 'Dagmar' and 'Genius' varieties are as well frost-resistant. Their poor performance in this test might be affected by the origin of seeds, in this case from organic trials.

## Keywords

Abiotic stress · winter hardiness · organic agriculture · *Triticum aestivum*

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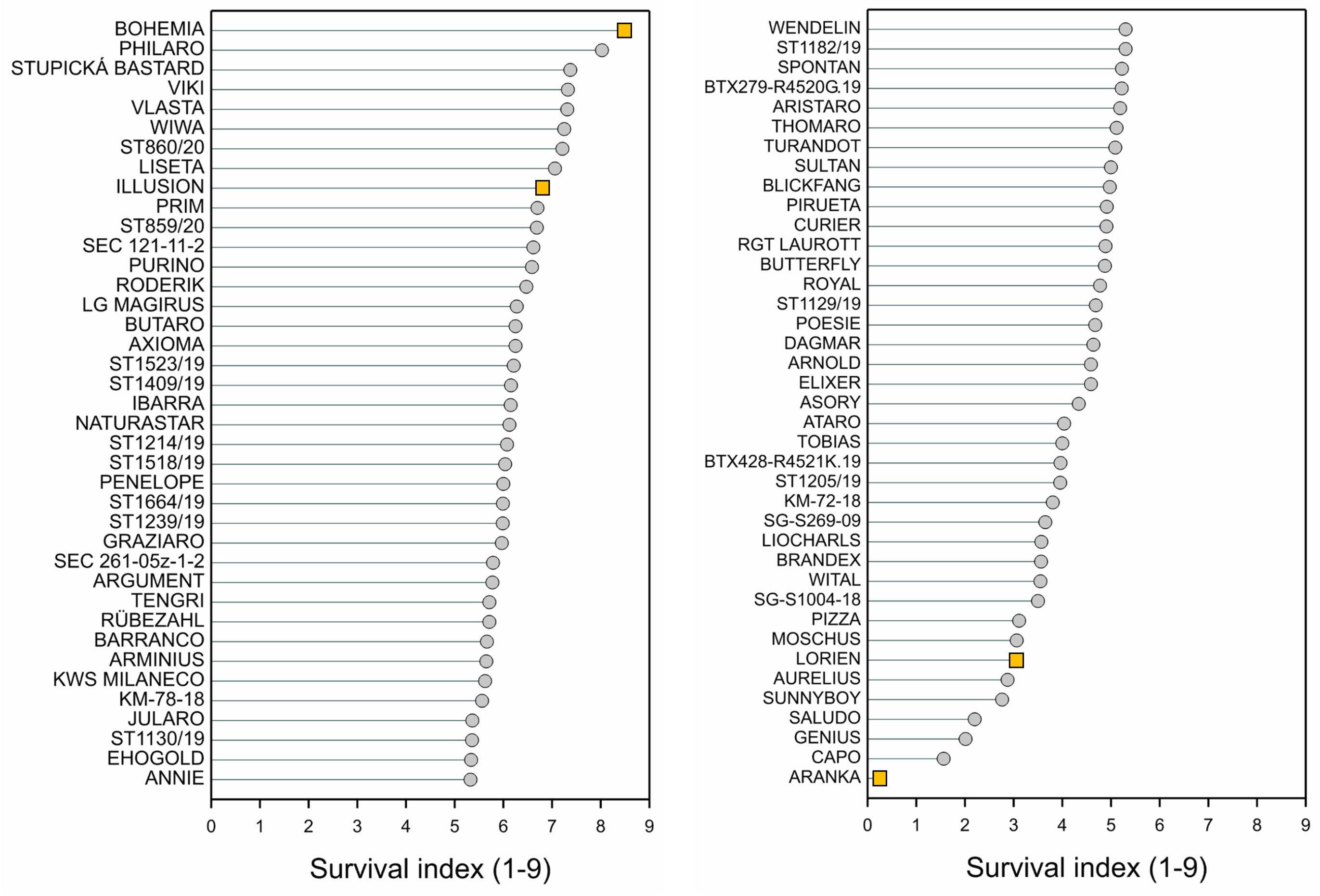

**Figure 1** Mean survival index of winter wheat varieties calculated across three freezing methods (KS, KU and L): 9 = 100% of plants survived; 0 = no plants survived; check varieties are indicated by a square symbol.

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# Genetic improvement of grain yield and associated traits in Norwegian spring wheat

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**Abstract**

Wheat cropping in Norway is challenged by several factors: severe winters limit winter wheat production and short vegetation period causes moderate yields compared to other European countries with more productive systems. The wet and windy climate, especially during late season causes delayed harvest in many years, and further promotes production challenges such as pre-harvest sprouting, lodging, and diseases like powdery mildew, Fusarium head blight and *Septoria nodorum* blotch. To deal with those limitations, experimental work in agriculture was initiated in Norway in 1889, quickly being followed by introducing artificial fertilizers and new varieties. From 1889 to 1962, wheat yields increased by approximately 13 kg·ha$^{-1}$ per year. From 1960 to 1974, spring wheat yields were increased further by approximately 130 kg·ha$^{-1}$ per year and the 1960-1992 period yielded in total an annual increase of 74 kg·ha$^{-1}$ per year. However, little is known about recent yield progress in Norwegian spring wheat cropping.

We assembled a collection of 21 spring wheat varieties released in Norway, covering the historically most widely cultivated material since 1972 as well as the current and just recently released varieties. It represents the breeding progress achieved from 1972 (onset of modern wheat cultivation in Norway) to the present day. The year when a variety was officially approved by the Plant Variety Board (year of release) was used to place it on the timeline. The collection was assembled to maximize its relevance for the actual market situation over the period.

We conducted yield trials with the collected varieties in field seasons 2016 – 2020 at Vollebekk Research Station (Ås, south-eastern Norway). This location represents the most important southern wheat cropping region in the country. To evaluate the effect of fertilization rates on yield performance and physiological traits, two N levels (managements) were applied before sowing: 75 kgN·ha$^{-1}$ and 150 kgN·ha$^{-1}$ of compound NPK fertilizer (referred later to as lowN and highN, respectively). The highN treatment reflects typical fertilization levels for spring wheat in Norway nowadays, while the lowN treatment was included to assess the performance of the varieties under less intensive management.

Following the seeding, standard local agronomic practice was followed to keep the trial plots free of weeds and plant diseases by use of herbicides and fungicides at recommended doses according to needs. Varieties were evaluated for grain yield, plant height, heading and physiological maturity, test weight, thousand kernel weight, grains per spike, grain protein content, and above-ground biomass at maturity. Based on these variables, additional parameters such as protein yield, harvest index, grains per area and spikes per area were derived.

Achieved grain yields in the representative seasons (6.01, 5.81, 5.70 and 5.35 Mg·ha$^{-1}$ in seasons 2016, 2017, 2019 and 2020) were significantly higher than the national long-term average of approximately 4.5 Mg·ha$^{-1}$. The highest-yielding variety in the collection was Arabella (released in 2014, 6.5 Mg·ha$^{-1}$) and the lowest-yielding was Runar (released in 1972, 5.2 Mg·ha$^{-1}$). Significant ($p < 0.05$) annual genetic gains in grain yield over the 1972-2019 period were observed for all the trial years and their mean except for field season 2017. Estimated annual genetic gain in grain yield vary from 16 kg·ha$^{-1}$ (0.33%) to 23.1 kg·ha$^{-1}$ (0.47%), averaging to 17.8 kg·ha$^{-1}$ (0.34%) per year. Genetic gains over the period were observed for both fertilization levels: 21.2 kg·ha$^{-1}$ (0.37%) and 14.3 kg·ha$^{-1}$ (0.32%) per year for highN and lowN, respectively (Figure 1).

No significant ($\alpha = 0.05$) interaction among genotypes and fertilization levels were observed for grain yield, giving means to conclude that breeding progress does not depend on intensive management. Increased fertilization has a significant positive effect on grain yield, plant height, protein content, plant biomass, grains per spike, thousand kernel weight, test weight, harvest index and days to maturity. Days to heading and number of spikes per area are the only traits unaffected by fertilization. None of the traits are subjects to a significant ($\alpha = 0.05$) genotype × management interaction. Grain yield in the collection is associated with number of grains per spike and number of grains per area. Breeding led to a development of later-maturing varieties with prolonged grain filling period, producing more grains per spike and grains per area. Grain yield gains have been driven by prolonged grain filling period and increasing the number of kernels per spike and number of kernels per area.

The annual genetic gains in grain yield (17.8 kg·ha$^{-1}$, 0.34%) show similarity to gains determined in numerous collections worldwide. The grain yield gains can be seen under both high and low fertilization inputs with similar cultivar ranking, showing that the source of improvement is of genetic nature. The new cultivars under low nitrogen input nearly approach the old ones under high input in

Mróz T, Dieseth JA, Lillemo M (2022) Historical grain yield genetic gains in Norwegian spring wheat under contrasting fertilization regimes. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 72. Jahrestagung 2021, 22-24 November, Raumberg-Gumpenstein, pp 41-42. University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-96-1

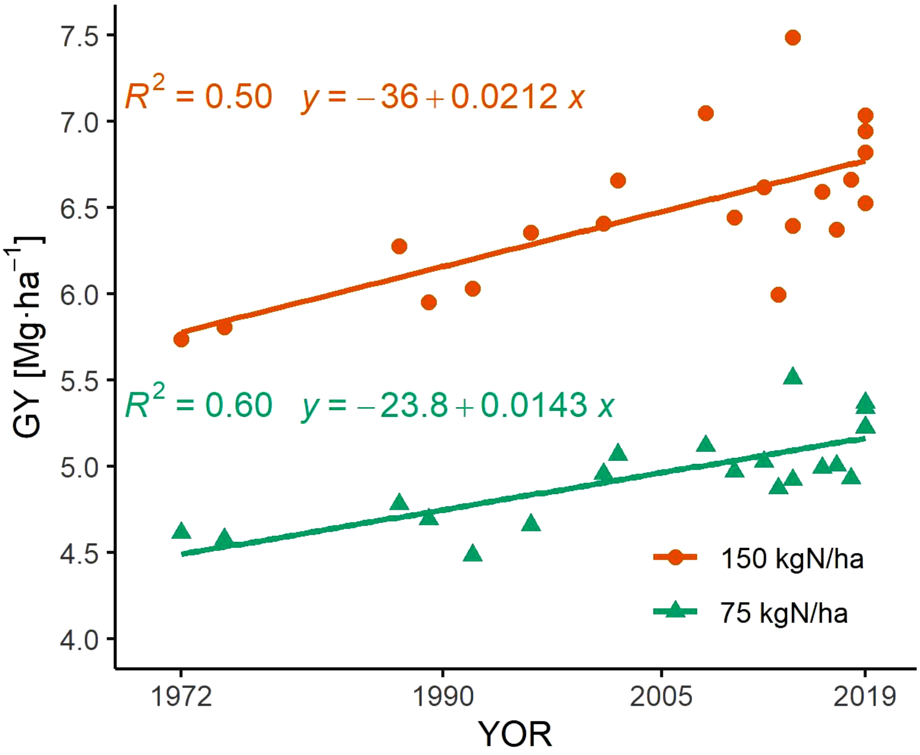

**Figure 1** Grain yield genetic gains over the 1972 – 2019 period under 75 kgN·ha$^{-1}$ (green, triangles) and 150 kgN·ha$^{-1}$ (red, dots) fertilization regimes. GY, grain yield; YOR, year of release

terms of grain yield, which underlines genetic contribution to yield progress. In our study, grain yield is correlated with the number of grains per spike and kernel weight (under low fertilization input) and with the number of grains per area (under high input), which align well with other studies on European wheat. New varieties take advantage of prolonged growing season in Norway (due to earlier spring, by approximately 4 days since 1970).

Those results underline the contribution of breeding to a more sustainable agriculture with lower environmental impact and increasing yields by introducing more efficient varieties which can also thrive under less intensive cultivation conditions.

### Keywords

Agricultural systems · crop management · nitrogen fertilization · plant and environment interactions · seed quality · wheat improvement

### Acknowledgements

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# Multi-environment trials of the ECOBREED wheat diversity panel

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## Abstract

The Horizon 2020 project ECOBREED aims to strengthen and/or initiate organic wheat breeding especially in European countries where the organic sector is still underrepresented. Two winter wheat nurseries were hitherto tested in multi-location trials in 2020 and 2021. The early maturity panel with 80 genotypes was tested in Slovakia, Hungary, Slovenia, Serbia and Romania, the late maturity panel with 60 genotypes in Germany, Czechia and Slovakia. The tested germplasm originates from France, Switzerland, Germany, Austria, Czechia, Slovakia, Hungary, Slovenia, Croatia, Serbia and Romania. Statistical analyses of the individual trials revealed partly large spatial effects along the rows or columns of the trial layout. Adjusted means were submitted in a second step to a sites regression analysis (GGE biplot). In addition, a floating check (*i.e.*, site highest yield) was calculated for each individual environment and finally the average yield performance relative to this check was calculated.

## Keywords

Floating check · genotype by environment interaction · grain yield · organic breeding · sites regression analysis · *Triticum aestivum*

## Introduction

Cereals occupy the largest organic crop acreage in Europe with 3 million hectares or 2.4% of the total cereal area. With 1.1 million hectares, wheat is the most important cereal (Busacca *et al*., 2022). Although several organic wheat breeding programs are already active (Wolfe *et al*., 2008), most of the organic wheat acreage is cultivated with varieties created in conventional breeding programs and only a few countries have established separate or supplementary organic value for cultivation and use (VCU) trials for wheat (Pedersen, 2012; Kovacs & Pedersen, 2018). The Horizon 2020 project ECOBREED (www.ecobreed.eu) aims to strengthen and/or initiate organic wheat breeding in Europe, especially in countries where the organic sector is still underrepresented. Therefore, multi-environment trials with winter wheat are carried out since 2020 in seven European countries. Here we report results on grain yield for the first two years.

## Material and methods

### *Plant material*

In total 137 winter wheat varieties, breeding lines and/or genetic resources were sourced from project partners and partners outside the consortium. The germplasm originates from Austria (10), Croatia (3), Czechia (17), France (8), Germany (28), Hungary (17), Romania (16), Serbia (7), Slovakia (19), Slovenia (4) and Switzerland (8). The material was divided into a late and early maturity group, including 60 and 80 genotypes, respectively (Table 1 and Table 2). Four varieties (*i.e.*, 'Arnold', 'Aurelius', 'Capo', 'Ehogold') were included as checks in both nurseries.

### *Test environments*

The late maturity nursery was cultivated in Feldkirchen (Germany), Uhrineves (Czechia) and Piešťany (Slovakia), the early maturity trial in Piešťany (Slovakia), Martonvásár (Hungary), Murska Sobota (Slovenia), Novi Sad (Serbia) and Fundulea (Romania). The field trials were laid out in most cases as randomized complete block designs, at some locations 'systematic designs' with uniformly arranged checks were used.

### *Statistical analysis*

In a first step the individual field trials were analysed for spatial trends according to their row-column arrangement using the auto-

Grausgruber H, Megyeri M, Hauptvogel P, Fusková M, Marinciu CM, Şerban G, Bauer N, Mayer M, Veskrna O, Jockovic B, Titan P, Meglič V (2022) Multi-environment trials of the ECOBREED wheat diversity panel. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 72. Jahrestagung 2021, 22-24 November, Raumberg-Gumpenstein, pp 43-52. University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-96-1

matic spatial analysis tool within the Mixed Models (REML) procedure of Genstat 21st ed. (VSNi Ltd., Hemel Hempstead). Efficiency of the best random model against the randomized complete block design was calculated as 1 - $SED_{best}/SED_{RCBD}$, where $SED_{best}$ is the standard error of differences of the best random model and $SED_{RCBD}$ that of the block design. Best linear unbiased estimators (BLUEs) for each genotype by environment (G×E) combination were thereafter submitted to a sites regression (GGE biplot) analysis according to Yan *et al*. (2000). In addition, the 'site highest yield' was applied as floating check to the G×E matrix. Thereby absolute values were transformed into relative values with the highest performance having always a value of 100 and automatically adjusting to the fertility level of each environment (Jensen, 1976). The site highest yield index (SHY) was finally calculated as a mean of the relative values across all environments. A maximum possible SHY index of 100 would mean that a genotype would have exhibited the highest grain yield in all environments.

## Results and discussion

### *Test environments*

Spatial analysis revealed different best random models for the individual test sites. For the late maturity panel, the best random model was more efficient than the RCBD in each test environment (7.3-54.9%). For the early maturity panel efficiency of the best random model was similarly high (9.6-57.2%) except for two environments (*i.e*. Serbia and Slovenia 2021) where the spatial model did not significantly improve trial efficiency. Cullis & Gleeson (1991) and Grondona *et al*. (1996) have shown that there is no one model that best fits all field trials, but that there is a high need of a two-dimensional (*i.e*. in the direction of the rows and columns) first order autoregressive model (AR1×AR1) even when the plot shape is rectangular. Contrary to these findings, in the present study an AR1×AR1 model was best in only two out of 16 test environments while an AR1 model in the direction of only columns was present in five test environments (Table S1). However, as recently Piepho *et al*. (2022) stated "*Sometimes, the large number of modeling options for spatial analysis may raise the false impression that design does not matter, and that a sophisticated analysis takes care of everything. Nothing could be further from the truth.*", it must be noted that in some trials the field design was not optimal to account best for natural or extraneous variation.

Grain yields were generally higher in 2021 compared to 2020. In the latter year, severe drought in Romania and Slovenia resulted in very low yields of less than 2 t·ha$^{-1}$ (Fig. 1, Table S3). The grain yields corresponded well to the documented national wheat productivity under organic conditions with the exception of Serbia. Yields in Novi Sad were more than double the mean productivity reported for Serbia (*e.g*. 4.9 t·ha$^{-1}$ in 2020, FAOSTAT). On the one hand, the chernozem soils of the Vojvojdina region are highly productive (Pavlović *et al*., 2017) which would explain higher yields compared to the rest of the country. On the other hand, the respective field is not a long-term organically managed field but a conventional field where no mineral fertilization was applied for this experiment. Therefore, a significant legacy effect of previous fertilization can be assumed. Worth mentioning is also that in each individual environment the overall mean across all genotypes is very similar to the mean of the four check varieties.

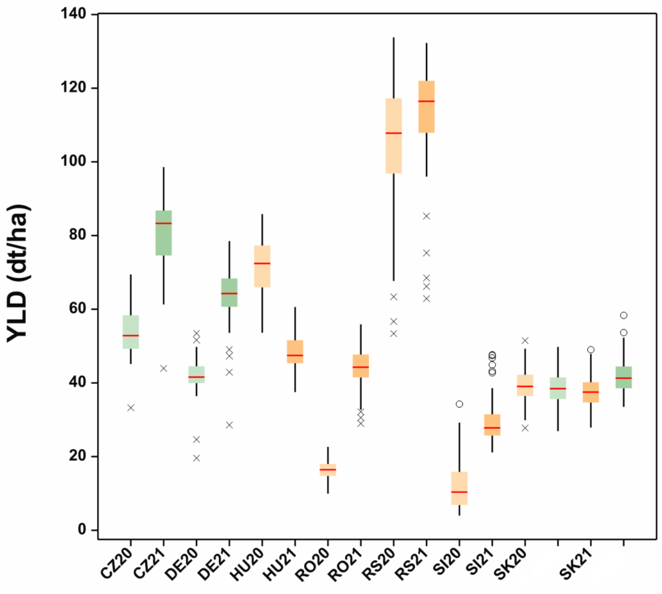

**Figure 1** Variation in grain yield of the ECOBREED winter wheat diversity panel. Green and orange boxes represent the late and early maturity nursery, respectively; environments are indicated by ISO country codes and year abbreviations.

### *Germplasm*

Similar variation in grain yield was observed in both the early and late nursery as demonstrated for the Slovakian site where both trials were grown (Fig. 1). For the late maturity group the highest SHY indices were calculated for 'Elixer', 'Turandot' and 'Liseta' and other varieties derived from conventional breeding programs (Table 1), while the best varieties released after organic VCU trials were 'Wendelin' and 'Naturastar', both coming out of a breeding program for organic agriculture (BFOA). Varieties developed in organic breeding programs (*e.g*. 'Graziaro', 'Aristaro' or 'Wiwa') were mostly performing below average. The lowest yield performance was recorded for the old variety 'Stupicka Bastard' (1927-1959). Generally, the results are in good agreement with those reported for Bavaria (LfL, 2021). Despite the generally negative relationship between yield performance and baking quality it is noteworthy that two varieties (*i.e*. 'Viki' and 'Wendelin') are among the top yielding varieties which are classified as baking quality E. The two organic heterogeneous material (OHM) 'Brandex' and 'Liocharls' showed medium performance. For the early maturity group, the highest SHY indices were calculated for several Martonvásár varieties (Table 2), while the lowest yields were also observed for old varieties and/or landraces (*i.e*. 'Bánkúti 1201', 'A15', 'Slovenská 200' and 'Radošínska Karola' from Hungary, Romania and Slovakia, respectively). The included OHM 'Mv Elit' showed medium performance, similar to the two OHMs included in the late maturity group. Compared to the studies of Döring et al. (2015) and Weedon & Finckh (2019) in which composite cross populations (CCPs) showed similar to 15% lower yields compared to the highest yielding variety, the OHMs included in this study were significantly inferior to the best genotypes. However, it has to be noted that in the present study a significant higher number of genotypes and test sites were included.

### *Genotype × environment interaction*

For the late maturity group, the two axes of the GGE biplot explain more than 70% of the variability. From Fig. 2 it is obvious that the

**Table 1** Site highest yield index (SHY) for the late maturity group of the ECOBREED winter wheat diversity panel.

| Genotype | SHY[1] | Genotype | SHY | Genotype | SHY | Genotype | SHY |
|---|---|---|---|---|---|---|---|
| Elixer | 93.1 | Moschus | 81.8 | Blickfang | 78.2 | Thomaro | 73.5 |
| Turandot | 93.0 | Ehogold | 81.6 | Tobias | 78.1 | Wital | 72.9 |
| Liseta | 91.1 | KWS Milaneco | 81.3 | SEC121-11-2 | 78.0 | KM72-18 | 72.1 |
| SG-S1004-18 | 87.9 | Sultan | 81.2 | BTX428-4521K.19 | 77.8 | Butaro | 71.9 |
| Asory | 87.9 | Genius | 81.2 | Roderik | 77.5 | Poesie | 71.7 |
| Viki | 85.5 | Purino | 81.0 | Liocharls | 77.5 | Pizza | 71.5 |
| Argument | 85.2 | Aurelius | 80.9 | Curier | 77.5 | Wiwa | 71.3 |
| Ibarra | 84.7 | Capo | 80.7 | Royal | 77.1 | Tengri | 71.2 |
| SG-S269-09 | 84.5 | Illusion | 80.2 | Spontan | 76.6 | Prim | 70.2 |
| KM78-18 | 84.3 | Graziaro | 79.7 | Arnold | 76.3 | Saludo | 69.6 |
| Penelope | 84.0 | Barranco | 79.4 | Arminius | 75.7 | Philaro | 69.0 |
| Wendelin | 83.4 | SEC261-05z-1-2 | 79.0 | Aristaro | 75.3 | BTX279-4520G.19 | 66.0 |
| Naturastar | 82.7 | Butterfly | 78.9 | Axioma | 74.3 | Pirueta | 65.9 |
| Annie | 82.5 | Dagmar | 78.8 | Jularo | 73.6 | Vlasta | 64.3 |
| Rübezahl | 82.3 | Brandex | 78.6 | Ataro | 73.5 | Stupická Bastard | 60.3 |

[1] SHY based on six environments, *i.e*. DE20, DE21, CZ20, CZ21, SK20 and SK21

**Table 2** Site highest yield index (SHY) for the early maturity group of the ECOBREED winter wheat diversity panel.

| GEN | SHY[1] | GEN | SHY | GEN | SHY | GEN | SHY |
|---|---|---|---|---|---|---|---|
| Mv Káplár | 90.6 | FDL Miranda | 76.2 | Juno | 73.0 | Ehogold | 69.5 |
| Mv Kepe | 88.0 | Genoveva | 76.0 | Anapurna | 72.9 | Stanislava | 68.9 |
| Mv Karéj | 86.1 | Alex | 75.6 | Goroljka | 72.8 | Edelmann | 68.6 |
| Mv Ménrót | 85.9 | NS Ilina | 75.6 | Nexera 923 | 72.8 | Mv Suba | 68.6 |
| Mv Kolompos | 85.0 | Ilona | 75.5 | BC Lira | 72.8 | Tata Mata | 68.5 |
| CCB Ingenio | 82.1 | NS 40S | 75.3 | Adelina | 72.3 | IS Escoria | 68.0 |
| Illico | 81.6 | Mv Toborzó | 74.8 | NS Efrosinia | 72.3 | IS Agilis | 67.8 |
| Unitar | 81.4 | Vulkan | 74.5 | NS Frajla | 71.9 | Glosa | 67.7 |
| Mv Uncia | 80.9 | PS Puqa | 74.5 | Capo | 71.6 | PS Dobromila | 67.6 |
| FDL Abundant | 80.2 | Mv Karizma | 74.4 | IS Laudis | 71.3 | Izalco CS | 67.0 |
| Sofru | 80.0 | Mv Bojtár | 74.1 | IS Gordius | 71.2 | PS Kvalitas | 67.0 |
| Mv Lucilla | 79.8 | Savinja | 74.0 | Mv Elit CCP | 71.0 | Viola | 66.7 |
| NS Mila | 78.5 | PS Jeldka | 74.0 | Voinic | 70.9 | Bona Vita | 66.6 |
| Ursita | 77.9 | Pitar | 73.9 | Reska | 70.6 | IS Mandala | 65.0 |
| Simnic 60 | 76.7 | NS Obala | 73.9 | Izvor | 70.5 | FDL Amurg | 64.9 |
| Nikol | 76.7 | Mv Pántlika | 73.8 | Zvezdana | 70.2 | 11248G2-1 | 63.3 |
| Solehio | 76.6 | Aurelius | 73.8 | Mv Magdaléna | 70.0 | Bánkúti 1201 | 59.3 |
| IS Solaris | 76.3 | Mv Mente | 73.7 | Alessio | 69.9 | A15 | 57.0 |
| Semnal | 76.3 | Farinelli | 73.3 | Bertold | 69.7 | Slovenská 200 | 56.1 |
| Mv Kolo | 76.2 | Litera | 73.1 | Arnold | 69.7 | Radošínska Karola | 55.3 |

[1] SHY based on ten environments, *i.e*. SK20, SK21, HU20, HU21, SI20, SI21, RS20, RS21, RO20 and RO21

genotypic response on the Slovakian site was very similar in both test years, whereas the Czech and German site show a different pattern in the two years, in 2020 forming one "mega-environment" together with the Slovakian site, in 2021 showing a different response and resulting in even separate mega-environments. 'Elixer', 'Turandot', 'Liseta' and 'Asory' are the winning genotypes in the first mega-environment, which is in good agreement to the SHY. At the German and Czech site in 2021 a bulk of varieties, e.g., 'Naturastar', 'Wendelin', 'Capo', 'Ehogold' or 'Tobias' are grouped at the border of the polygon. Similar to the SHY, the old variety 'Stupická Bastard' and 'Vlasta' are significantly inferior in performance, taking the most extreme positions on the left part of the polygon.

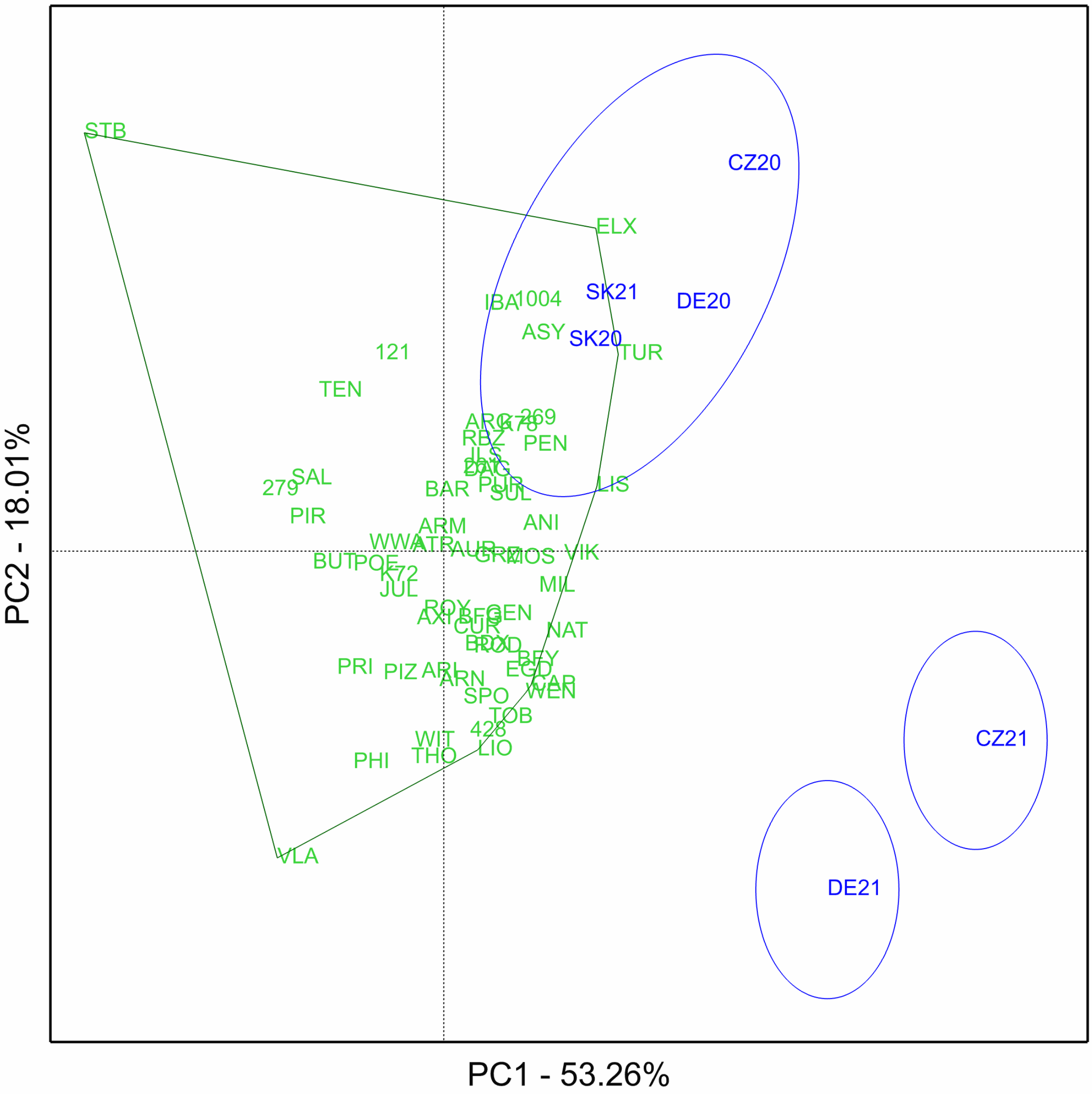

**Figure 2** GGE biplot of the late maturity winter wheat nursery. Environments are printed in blue, codes represent country code and test year, and mega-environments are encircled. Genotypes are printed in green and abbreviated in most cases by the first three letters; breeding lines by the first number of their code (see Table S2 for detailed abbreviations).

For the early maturity group, the two axes of the GGE biplot explain more than 75% of the variability. Responses of the test environments were very similar with the exception of the Serbian site which has in both years a very extreme location on the plot (Fig. 3). The Slovenian and Slovakian site showed a very similar response in both test years. The severe drought observed 2020 in Romania resulted in a grouping of this test environment together with the Slovenian site which is characterized by the lowest productivity. In 2021, the Romanian site gave a response similar to the Hungarian site. As in the late maturity group, the GGE biplot confirms the results of SHY, identifying several varieties from the Martonvásár breeding programme as 'winning genotypes', together with a few varieties from Fundulea, Novi Sad and international breeding programmes. Old varieties and/or landraces included in the trial performed significantly inferior and can be found on the very left of the biplot.

Contrary to the late maturity group, only one variety (*i.e.* 'Edelmann') of the early maturity panel was released after an organic VCU test. However, 'Edelmann' was released in Austria under environmental conditions different to the prevalent test environments. Various studies (*e.g.* Oberforster et al., 2000; Kempf, 2003; Murphy et al., 2007; Reid et al., 2009; Baenziger et al., 2011; Kirk et al., 2012) have shown that it is not appropr--iate to use conventional trial results for direct selection of breeding lines/varieties for organic production. Organic VCU and/or post-registration trials can boost organic breeding programs and variety release as demonstrated for Austria (Löschenberger et al., 2008; AGES, 2021), Germany (Miedaner, 2018; BSA, 2021), Switzerland (Dierauer & Levy, 2021), and France (Rolland et al., 2014). Despite the satisfactory availability of 'organic varieties' in some countries, the greatest acreage of organic wheat is still cultivated with varieties from conventional breeding programs (Miedaner, 2018; AGES, 2021). The reason for that is most probably the yield penalty of 'organic' compared to 'conventional varieties' which is obvious in the late maturity group where conventional varieties were top performing, some BFOA varieties were above average and the organic varieties were medium to low performing. Miedaner (2018) argues that existing organic breeding programs are still small in size and, therefore, limited in genetic diversity. In addi-

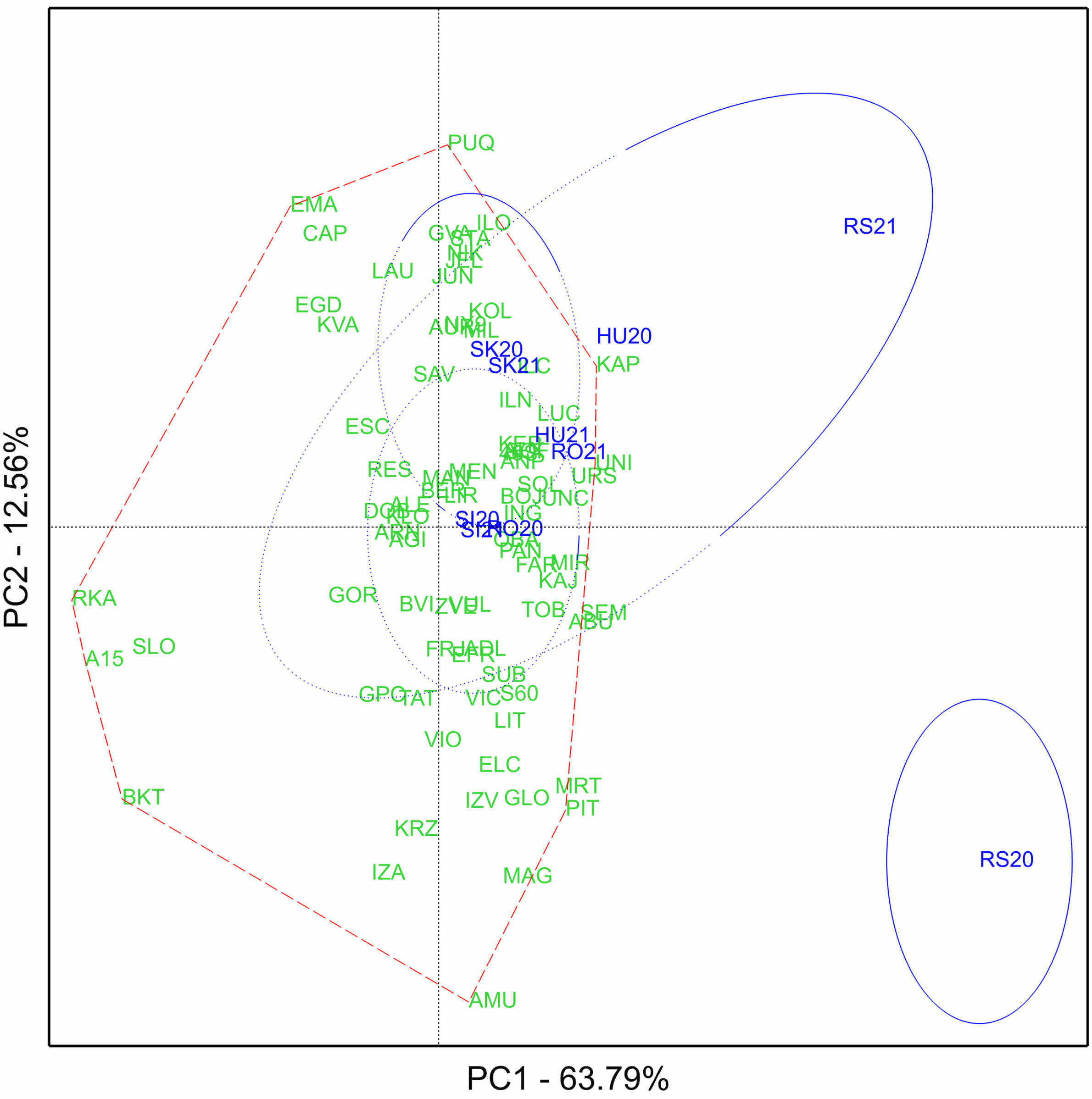

**Figure 3** GGE biplot of the late maturity winter wheat nursery. Environments are printed in blue, codes represent country code and test year, and mega-environments are encircled. Genotypes are printed in green and abbreviated in most cases by the first three letters; breeding lines by the first number of their code (see Table S3 for detailed abbreviations).

tion, it can be assumed that the trial network of these programs is smaller compared to conventional programs. Considering both facts response to selection is smaller.

## Conclusions

The European Union's Farm to Fork Strategy aims at a sustainable food production by amongst others reducing negative environmental impact and reverse the loss of biodiversity (EU, 2020). The reduction of pesticides and nutrient losses, highly nutritious food and a highly diverse agricultural landscape goes along with principles of organic agriculture. Therefore, an increase of organic crop production is part of the Farm to Fork Strategy. Although, several organic wheat breeding programs are already existing, mainly in Central Europe, and their output is satisfactory with respect to number of varieties their impact on the seed market and cultivation is still limited. Reasons therefor are manifold, from smaller sized breeding programs to smaller efforts in seed multiplication and distribution. Organic VCU tests, post-registration tests or farmers' trials are necessary to make 'organic breeding' or 'breeding for organic' more attractive to wheat breeders concerned about sustainable wheat production. In the ECOBREED project, wheat breeders from Germany, Czechia, Slovakia, Hungary, Serbia and Romania are involved which play a key role in their national seed market. Therefore, one aim of the project, *i.e.*, to increase the availability of organic seeds, can easily be reached. Moreover, in the ECOBREED wheat diversity panel a large genetic diversity is present in historic and currently important germplasm which can form the basis of future breeding programs for organic wheat growing in the individual countries. The first step was already made by crosses carried out by the partners. However, it is essential that the individual states enable also the testing of new breeding lines under organic conditions, either by a separate organic VCU test or by including organic sites and untreated trials in their ongoing conventional VCU testing.

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**APPENDIX**

Supplementary tables S1 (spatial models) , S2 (BLUEs late maturity group) and S3 (BLUEs early maturity group)

**Table S1** Best random spatial model and covariance structure defined for the random model in the individual test environments.

| Test site | Maturity panel | 2020 | | 2021 | |
|---|---|---|---|---|---|
| | | Random model[1] | Covariance[2] | Random model | Covariance |
| Feldkirchen, DE | Late | row.col | Id×AR1 | row.col | Id×Pow1 |
| Uhrineves, CZ | Late | row.col2D | Pow1 | rep+row.col | Id×AR1 |
| Piešťany, SK | Late | row.col | Id×AR1 | row.col | AR1×AR1 |
| Piešťany, SK | Early | rep.col+rep.row.col | Id×AR1×AR1 | row.col+units | AR1×AR1 |
| Martonvásár, HU | Early | row+row.col2D | Pow1 | row+row.col2D | Pow1 |
| Murska Sobota, SI | Early | row.col | Id×AR1 | row.col | Id×Id |
| Novi Sad, RS | Early | row.col | Id×Id | row.col | Id×Id |
| Fundulea, RO | Early | col | | row+col+row.col | Id×AR1 |

[1] row, rows; col, columns; rep, replication/block; units, nugget effect (measurement errors)

[2] Id, identity; AR1, first order auto-regression, Pow1; first order Power model (city-block)

**Table S2** Best linear unbiased estimators (BLUEs) for grain yield (dt·ha$^{-1}$) of the late maturity nursery in the respective location and year. Genotypes are ordered according to descending grand means; BLUEs were derived from the best random model.

| Genotype | Id[1] | DE20[2] | DE21 | CZ20 | CZ21 | SK20 | SK21 |
|---|---|---|---|---|---|---|---|
| Turandot | TUR | 49.7 | 72.9 | 69.5 | 91.3 | 43.2 | 53.7 |
| Elixer | ELX | 53.5 | 63.7 | 67.1 | 91.9 | 43.5 | 58.3 |
| Liseta | LIS | 51.6 | 78.5 | 64.0 | 87.0 | 47.6 | 43.1 |
| Asory | ASY | 47.1 | 62.1 | 59.0 | 86.5 | 48.6 | 52.3 |
| SG-S1004-18 | 1004 | 49.6 | 64.6 | 62.1 | 81.3 | 46.1 | 51.4 |
| Viki | VIK | 47.0 | 74.9 | 64.0 | 87.9 | 37.4 | 42.7 |
| SG-S269-09 | 269 | 46.5 | 64.0 | 62.3 | 86.9 | 41.4 | 45.4 |
| Penelope | PEN | 47.6 | 65.0 | 62.3 | 87.2 | 38.5 | 44.7 |
| Wendelin | WEN | 44.1 | 76.9 | 50.9 | 86.0 | 41.5 | 44.6 |
| Ibarra | IBA | 48.3 | 67.0 | 67.5 | 74.2 | 39.0 | 47.7 |
| KM78-18 | K78 | 45.4 | 67.3 | 61.8 | 81.0 | 44.5 | 43.8 |
| Naturastar | NAT | 46.7 | 65.6 | 53.6 | 96.9 | 40.4 | 40.2 |
| Argument | ARG | 49.8 | 67.4 | 51.3 | 75.9 | 49.8 | 47.6 |
| Annie | ANI | 42.9 | 60.3 | 57.0 | 94.9 | 43.3 | 42.3 |
| KWS Milaneco | MIL | 40.7 | 61.1 | 59.4 | 98.6 | 41.3 | 38.0 |
| Moschus | MOS | 43.9 | 69.9 | 58.9 | 84.3 | 37.1 | 43.8 |
| Capo | CAP | 41.6 | 69.7 | 55.8 | 92.9 | 37.5 | 39.7 |
| Ehogold | EGD | 42.9 | 70.6 | 48.1 | 88.3 | 38.1 | 49.1 |
| Sultan | SUL | 45.7 | 61.3 | 55.0 | 87.7 | 36.7 | 47.9 |
| Rübezahl | RBZ | 45.0 | 64.2 | 55.8 | 79.0 | 43.8 | 46.3 |
| Genius | GEN | 42.2 | 69.1 | 51.2 | 84.4 | 42.3 | 44.4 |
| Purino | PUR | 45.0 | 63.1 | 57.2 | 83.8 | 39.6 | 43.6 |
| Butterfly | BFY | 42.0 | 67.1 | 58.3 | 92.0 | 33.3 | 38.0 |
| Illusion | ILS | 41.5 | 61.8 | 61.9 | 82.8 | 41.9 | 39.5 |
| Graziaro | GRZ | 42.6 | 65.1 | 54.4 | 84.2 | 35.2 | 47.2 |
| Aurelius | AUR | 42.4 | 72.5 | 56.4 | 73.3 | 46.5 | 37.7 |
| Dagmar | DAG | 38.6 | 59.3 | 60.9 | 85.1 | 38.4 | 43.1 |
| Tobias | TOB | 40.0 | 64.4 | 47.4 | 93.1 | 38.8 | 41.5 |

**Table S2** Continued.

| Genotype | Id[1] | DE20[2] | DE21 | CZ20 | CZ21 | SK20 | SK21 |
|---|---|---|---|---|---|---|---|
| Brandex | BDX | 40.8 | 66.2 | 49.2 | 85.2 | 38.5 | 44.6 |
| SEC261-05z-1-2 | 261 | 44.1 | 54.9 | 56.8 | 88.4 | 42.4 | 37.8 |
| BTX428-R4521K.19 | 428 | 41.2 | 76.5 | 52.7 | 79.0 | 33.0 | 41.0 |
| Barranco | BAR | 41.6 | 65.1 | 52.2 | 74.8 | 39.3 | 50.1 |
| Liocharls | LIO | 40.7 | 71.4 | 50.2 | 85.6 | 37.8 | 36.9 |
| Blickfang | BFG | 42.1 | 67.0 | 52.8 | 82.0 | 38.0 | 40.6 |
| Roderik | ROD | 41.2 | 65.4 | 54.9 | 86.7 | 38.5 | 35.4 |
| Curier | CUR | 38.5 | 63.9 | 51.2 | 85.0 | 40.5 | 41.1 |
| Spontan | SPO | 41.0 | 67.7 | 51.1 | 85.0 | 33.9 | 40.0 |
| Royal | ROY | 42.6 | 71.2 | 54.1 | 72.3 | 38.0 | 37.6 |
| Arnold | ARN | 40.0 | 64.0 | 47.3 | 84.5 | 41.5 | 37.6 |
| SEC121-11-2 | 121 | 41.7 | 60.9 | 58.4 | 65.7 | 44.2 | 42.6 |
| Arminius | ARM | 40.5 | 56.6 | 52.9 | 82.5 | 39.2 | 39.7 |
| Aristaro | ARI | 40.7 | 68.8 | 50.6 | 76.7 | 35.3 | 38.6 |
| Axioma | AXI | 37.5 | 58.8 | 49.1 | 84.0 | 34.5 | 44.0 |
| Ataro | ATR | 39.5 | 53.6 | 53.5 | 85.0 | 34.0 | 39.5 |
| Thomaro | THO | 39.1 | 69.0 | 46.4 | 78.1 | 36.3 | 35.7 |
| Wital | WIT | 42.1 | 71.7 | 50.9 | 74.9 | 26.9 | 37.3 |
| Jularo | JUL | 36.9 | 62.0 | 49.4 | 74.4 | 36.1 | 43.7 |
| KM72-18 | K72 | 41.9 | 60.8 | 54.0 | 73.8 | 31.0 | 36.1 |
| Pizza | PIZ | 37.3 | 60.6 | 46.8 | 79.0 | 31.5 | 41.5 |
| Wiwa | WWA | 41.3 | 53.8 | 51.7 | 77.8 | 32.8 | 36.6 |
| Poesie | POE | 41.2 | 57.4 | 47.7 | 73.2 | 33.8 | 40.5 |
| Butaro | BUT | 40.7 | 63.7 | 45.2 | 61.3 | 38.1 | 41.1 |
| Tengri | TEN | 39.7 | 47.3 | 51.2 | 71.4 | 35.9 | 43.5 |
| Prim | PRI | 36.7 | 63.2 | 45.4 | 69.3 | 35.0 | 38.4 |
| Philaro | PHI | 36.5 | 67.9 | 47.1 | 70.4 | 31.3 | 33.5 |
| Saludo | SAL | 39.9 | 54.0 | 48.2 | 63.8 | 36.3 | 39.1 |
| Pirueta | PIR | 24.7 | 42.9 | 49.7 | 76.8 | 38.5 | 39.6 |
| BTX279-R4520G.19 | 279 | 39.2 | 49.1 | 47.6 | 64.3 | 31.7 | 36.8 |
| Vlasta | VLA | 19.6 | 59.7 | 33.3 | 72.4 | 40.1 | 41.4 |
| Stupická Bastard | STB | 36.8 | 28.6 | 48.2 | 44.0 | 37.6 | 39.0 |
| SED[3] |  | 2.4 | 3.5 | 3.9 | 5.0 | 4.3 | 3.6 |

[1] Genotype Id (used in Fig. 2); [2] Environment: combination of ISO country codes and year; [3] Standard error of difference

**Table S3** Best linear unbiased estimators (BLUEs) for grain yield (dt·ha$^{-1}$) of the early maturity nursery in the respective location and year. Genotypes are ordered according to descending grand means; BLUEs were derived from the best random model.

| Genotype | Id[2] | SK20[3] | SK21 | HU20 | HU21 | SI20 | SI21 | RS20 | RS21 | RO20 | RO21 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mv Káplár | KAP | 42.8 | 45.0 | 85.9 | 53.4 | 24.0 | 43.2 | 125.3 | 131.1 | 20.9 | 54.7 |
| Mv Karéj | KAJ | 40.9 | 38.4 | 76.0 | 48.0 | 27.8 | 47.5 | 122.7 | 119.9 | 16.4 | 56.0 |
| Mv Ménrót | MRT | 37.7 | 38.4 | 73.1 | 48.0 | 26.0 | 46.8 | 131.9 | 115.5 | 20.2 | 52.5 |
| Mv Kepe | KEP | 44.4 | 39.8 | 71.3 | 51.8 | 34.3 | 44.9 | 112.2 | 121.8 | 19.9 | 48.4 |
| Unitar | UNI | 41.2 | 40.3 | 78.7 | 54.5 | 7.0 | 27.6 | 127.6 | 132.3 | 22.7 | 53.8 |
| Mv Kolompos | KOL | 38.6 | 43.1 | 81.5 | 52.9 | 29.3 | 47.7 | 103.4 | 121.1 | 17.3 | 41.6 |
| Illico | ILC | 46.7 | 44.4 | 73.4 | 52.8 | 20.0 | 36.0 | 114.1 | 126.5 | 14.8 | 45.9 |
| Mv Uncia | UNC | 36.5 | 37.8 | 76.1 | 55.8 | 15.3 | 32.8 | 119.6 | 125.1 | 19.3 | 54.6 |
| FDL Abundant | ABU | 39.7 | 39.2 | 79.5 | 50.7 | 12.8 | 28.0 | 129.4 | 123.0 | 22.3 | 47.2 |
| CCB Ingenio | ING | 45.7 | 36.6 | 79.0 | 56.2 | 21.3 | 38.3 | 116.7 | 116.7 | 18.0 | 42.1 |
| Mv Lucilla | LUC | 47.3 | 35.1 | 84.4 | 47.4 | 15.0 | 30.2 | 118.3 | 125.8 | 19.0 | 46.9 |
| Ursita | URS | 42.8 | 38.4 | 80.1 | 47.4 | 9.7 | 27.4 | 124.9 | 130.5 | 18.4 | 49.0 |
| Solehio | SOL | 49.3 | 38.7 | 75.3 | 54.9 | 8.3 | 23.2 | 130.4 | 123.6 | 14.5 | 47.6 |
| Semnal | SEM | 40.8 | 35.0 | 77.7 | 54.2 | 5.8 | 29.9 | 130.4 | 127.4 | 17.9 | 44.7 |
| Sofru | SOF | 43.3 | 49.0 | 77.0 | 57.1 | 12.8 | 23.0 | 116.3 | 116.2 | 19.1 | 48.8 |
| FDL Miranda | MIR | 38.1 | 41.8 | 77.6 | 51.5 | 5.3 | 24.7 | 124.7 | 124.5 | 21.2 | 44.5 |
| NS Ilina | ILN | 42.3 | 46.1 | 78.5 | 53.0 | 9.0 | 28.9 | 113.0 | 120.9 | 13.2 | 44.5 |
| Pitar | PIT | 34.5 | 30.7 | 78.6 | 51.2 | 8.5 | 32.7 | 133.8 | 119.8 | 17.1 | 41.8 |
| Alex | ALX | 38.9 | 35.3 | 82.9 | 54.2 | 10.2 | 28.4 | 113.3 | 120.9 | 17.2 | 45.6 |
| NS Mila | MIL | 51.5 | 40.2 | 71.0 | 52.4 | 11.0 | 27.2 | 105.0 | 120.6 | 20.6 | 47.0 |
| Simnic 60 | S60 | 39.9 | 39.4 | 83.8 | 47.2 | 13.5 | 26.1 | 122.9 | 107.3 | 19.2 | 46.1 |
| Farinelli | FAR | 41.5 | 41.5 | 73.3 | 57.0 | 8.3 | 24.9 | 120.6 | 118.0 | 10.8 | 47.8 |
| NS 40S | 40S | 39.8 | 39.5 | 72.9 | 49.4 | 9.3 | 27.7 | 112.6 | 123.8 | 17.4 | 49.6 |
| Ilona | ILO | 33.4 | 42.7 | 85.3 | 52.9 | 4.0 | 27.2 | 100.9 | 125.7 | 20.0 | 49.5 |
| Nikol | NIK | 41.6 | 42.5 | 78.2 | 60.6 | 10.9 | 24.5 | 99.2 | 118.7 | 17.2 | 47.7 |
| IS Solaris | SOL | 46.7 | 39.8 | 75.4 | 47.5 | 9.0 | 32.1 | 106.5 | 118.6 | 17.4 | 47.9 |
| Mv Toborzó | TOB | 30.7 | 33.9 | 68.5 | 50.4 | 14.3 | 30.7 | 118.4 | 126.2 | 17.3 | 50.3 |
| Anapurna | ANP | 43.8 | 47.9 | 72.2 | 46.4 | 11.3 | 21.2 | 115.7 | 122.3 | 11.7 | 43.8 |
| Mv Bojtár | BOJ | 39.7 | 36.0 | 72.7 | 49.4 | 9.5 | 24.8 | 113.8 | 123.7 | 18.4 | 47.6 |
| NS Obala | OBA | 41.0 | 42.5 | 71.1 | 52.8 | 6.3 | 25.0 | 115.9 | 118.1 | 16.8 | 45.8 |
| Mv Pántlika | PAN | 36.6 | 35.7 | 78.1 | 46.1 | 14.0 | 29.4 | 115.5 | 121.5 | 16.6 | 41.2 |
| Genoveva | GVA | 44.7 | 46.3 | 79.2 | 46.9 | 5.5 | 31.7 | 96.5 | 117.3 | 18.0 | 48.4 |
| PS Puqa | PUQ | 43.0 | 38.4 | 80.3 | 43.5 | 7.5 | 27.3 | 94.3 | 127.9 | 17.8 | 51.9 |
| PS Jeldka | JEL | 44.9 | 41.7 | 73.4 | 53.4 | 9.8 | 25.2 | 99.3 | 121.9 | 14.1 | 47.3 |
| Litera | LIT | 31.0 | 34.5 | 81.9 | 49.3 | 4.5 | 28.0 | 120.2 | 109.2 | 20.2 | 50.8 |
| Vulkan | VUL | 41.5 | 41.0 | 70.7 | 45.4 | 17.3 | 31.1 | 111.5 | 110.5 | 14.2 | 44.0 |
| Nexera 923 | NX9 | 45.5 | 40.2 | 71.6 | 49.4 | 13.7 | 24.4 | 101.3 | 122.4 | 13.8 | 40.7 |
| Aurelius | AUR | 41.2 | 34.0 | 75.5 | 51.7 | 14.3 | 25.7 | 97.4 | 118.8 | 16.4 | 47.8 |
| Juno | JUN | 41.6 | 40.0 | 72.4 | 51.1 | 7.5 | 31.4 | 96.6 | 122.9 | 16.0 | 42.6 |
| Mv Mente | MEN | 38.9 | 39.4 | 65.5 | 45.7 | 14.3 | 28.4 | 105.0 | 121.3 | 18.0 | 43.8 |
| BC Lira | LIR | 38.4 | 40.5 | 69.6 | 50.7 | 11.3 | 26.3 | 106.0 | 115.2 | 15.6 | 46.5 |
| Adelina | ADL | 37.5 | 37.3 | 69.6 | 47.8 | 11.5 | 25.7 | 113.9 | 113.4 | 17.8 | 43.6 |
| Savinja | SAV | 39.7 | 37.7 | 72.5 | 45.4 | 17.0 | 30.5 | 96.7 | 116.9 | 16.3 | 45.4 |
| Mv Kolo | KLO | 36.2 | 33.9 | 75.4 | 46.8 | 27.0 | 38.6 | 96.9 | 107.4 | 17.5 | 37.8 |
| NS Efrosinia | EFR | 39.9 | 39.6 | 61.5 | 51.5 | 7.0 | 25.9 | 112.5 | 112.0 | 18.5 | 46.4 |
| Mv Magdaléna | MAG | 32.1 | 32.2 | 64.4 | 46.7 | 5.5 | 31.6 | 125.6 | 114.8 | 17.9 | 43.5 |
| Mv Elit CCP | ELC | 29.9 | 29.7 | 65.2 | 42.4 | 17.5 | 31.5 | 116.9 | 117.6 | 14.9 | 48.1 |
| Izvor | IZV | 30.8 | 35.7 | 65.0 | 48.6 | 5.8 | 34.4 | 117.6 | 109.4 | 15.4 | 49.8 |
| Voinic | VIC | 39.0 | 36.4 | 63.9 | 46.0 | 10.3 | 26.7 | 115.6 | 114.6 | 16.6 | 42.7 |
| Glosa | GLO | 30.5 | 31.1 | 74.3 | 51.3 | 6.0 | 25.0 | 123.7 | 115.4 | 15.2 | 37.1 |
| Stanislava | STA | 37.8 | 36.8 | 73.6 | 45.3 | 5.8 | 25.8 | 96.7 | 131.8 | 14.4 | 41.1 |

**Table S3** Continued.

| Genotype | Id[2] | SK20[3] | SK21 | HU20 | HU21 | SI20 | SI21 | RS20 | RS21 | RO20 | RO21 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| NS Frajla | FRJ | 37.4 | 41.7 | 61.6 | 55.5 | 10.5 | 26.7 | 108.6 | 108.2 | 16.5 | 42.2 |
| Mv Karizma | KRZ | 37.5 | 31.5 | 68.9 | 44.6 | 27.3 | 34.1 | 109.4 | 96.0 | 16.4 | 42.4 |
| Mv Suba | SUB | 36.5 | 34.7 | 68.5 | 45.7 | 9.5 | 21.2 | 116.9 | 118.2 | 15.2 | 41.2 |
| IS Laudis | LAU | 43.2 | 46.8 | 71.8 | 47.8 | 6.5 | 26.7 | 89.3 | 111.6 | 15.0 | 44.1 |
| IS Gordius | GOR | 38.9 | 39.6 | 80.8 | 47.6 | 7.5 | 25.5 | 96.8 | 102.2 | 17.1 | 46.3 |
| Zvezda | ZVE | 35.0 | 35.1 | 68.0 | 44.2 | 12.9 | 23.2 | 107.0 | 114.5 | 18.3 | 42.9 |
| Bertold | BER | 35.8 | 37.8 | 71.2 | 45.2 | 5.0 | 29.7 | 101.4 | 115.7 | 18.2 | 40.7 |
| Alessio | ALE | 41.9 | 34.8 | 65.2 | 46.8 | 17.5 | 31.5 | 97.9 | 111.2 | 10.0 | 42.0 |
| Reska | RES | 33.4 | 31.1 | 68.9 | 38.3 | 18.5 | 33.3 | 90.6 | 112.2 | 16.3 | 47.9 |
| Capo | CAP | 42.3 | 42.2 | 72.4 | 41.9 | 19.9 | 35.6 | 76.7 | 106.2 | 11.8 | 40.4 |
| IS Agilis | AGI | 37.6 | 36.0 | 74.3 | 47.2 | 6.5 | 22.5 | 99.4 | 105.3 | 15.7 | 43.9 |
| Viola | VIO | 37.7 | 36.6 | 74.1 | 42.2 | 7.0 | 26.6 | 112.2 | 105.3 | 15.6 | 30.6 |
| Arnold | ARN | 36.7 | 37.1 | 65.5 | 38.5 | 16.5 | 29.4 | 95.7 | 110.6 | 16.5 | 40.6 |
| Tata Mata | TAT | 36.6 | 33.8 | 66.4 | 45.6 | 14.5 | 26.1 | 105.4 | 105.9 | 16.0 | 36.8 |
| IS Mandala | MAN | 36.8 | 37.8 | 64.2 | 46.3 | 6.0 | 25.6 | 101.2 | 122.1 | 12.1 | 32.2 |
| PS Dobromila | DOB | 39.9 | 36.2 | 64.1 | 47.3 | 10.3 | 28.5 | 94.1 | 107.6 | 13.0 | 41.2 |
| FDL Amurg | AMU | 27.8 | 27.9 | 58.6 | 38.0 | 11.0 | 22.9 | 123.3 | 111.3 | 16.3 | 43.9 |
| IS Escoria | ESC | 43.2 | 34.6 | 69.9 | 51.0 | 6.5 | 24.7 | 89.3 | 104.2 | 15.6 | 42.0 |
| Bona Vita | BVI | 34.0 | 29.6 | 72.5 | 44.9 | 63 | 27.4 | 101.1 | 110.6 | 18.8 | 35.6 |
| Goroljka | GOR | 39.1 | 36.7 | 56.7 | 44.0 | 23.8 | 42.8 | 88.0 | 85.3 | 15.8 | 44.7 |
| Ehogold | EGD | 43.1 | 38.3 | 67.8 | 45.3 | 17.3 | 26.7 | 77.6 | 103.7 | 14.3 | 41.4 |
| PS Kvalitas | KVA | 42.2 | 36.9 | 64.8 | 47.3 | 8.8 | 27.0 | 80.9 | 107.2 | 12.2 | 46.5 |
| Edelmann | EMA | 39.3 | 34.7 | 73.3 | 46.0 | 15.0 | 27.0 | 72.2 | 107.1 | 14.7 | 43.7 |
| Izalco CS | IZA | 32.9 | 30.0 | 57.0 | 39.7 | 17.8 | 32.0 | 105.5 | 100.2 | 14.6 | 42.2 |
| 11248G2-1 (FDLGPC1) | GPC | 32.8 | 35.6 | 60.4 | 46.1 | 5.0 | 26.6 | 98.6 | 102.6 | 12.4 | 41.2 |
| Bánkúti 1201 | BKT | 37.5 | 29.9 | 55.6 | 37.5 | 17.3 | 31.1 | 67.7 | 66.2 | 13.2 | 32.8 |
| Slovenská 200 | SLO | 35.6 | 31.2 | 53.6 | 43.0 | 6.8 | 28.7 | 63.4 | 75.3 | 11.0 | 34.4 |
| A15 | A15 | 30.6 | 31.3 | 63.5 | 41.5 | 6.0 | 28.9 | 56.7 | 62.9 | 15.2 | 38.5 |
| Radošínska Karola | RKA | 35.2 | 35.3 | 55.0 | 38.8 | 6.8 | 28.2 | 53.4 | 68.5 | 14.2 | 29.0 |
| SED[3] |  | 3.2 | 2.8 | 4.0 | 4.3 | 4.0 | 6.1 | 5.7 | 7.1 | 1.9 | 4.4 |

[1] Genotype Id (used in Fig. 3); [2] Environment: combination of ISO country codes and year; [3] Standard error of difference

72. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
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# Baking quality of wheat in organic farming

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## Abstract

Organic farming is characterised amongst others by the use of organic rather than mineral fertilizers. Organic cultivars should be able to perform at low levels of organic fertilizers, especially at a lower nitrogen level. In the present study, baking quality of wheat was evaluated in samples from both conventional and organic field trials.

The field trials were conducted from 2019 to 2021 in Stupice (low-input: 150 kg N·ha$^{-1}$; herbicide and seed treatment; high-input: 190 kg N·ha-1; herbicide, fungicide and seed treatment) and Uhřiněves (organic; weed management by harrowing) with 20 varieties of winter wheat. Pre-crops were usually legumes except in 2020 in Uhřiněves where it was potatoes. The following quality analyses were carried out on harvest samples: protein content and test weight by NIRS, Hagberg falling number, wet gluten content by the Glutomatic machine, SDS sedimentation value and baking volume by a mini rapid-mix-test (RMT).

In the present experiment, grain yield under organic farming was in average 35% to 40% lower compared to the conventional low-input and high-input trials, respectively. However, the yield penalty was higher in 2020 when the pre-crop was potato, and it was significantly less in 2021 when the lowest grain yields were recorded for the conventional trials but the highest yield for the organic trial. Potato as a pre-crop had also negative effects on the grain quality parameters (Table 1). Hence, a great influence of the pre-crop on yield and grain quality was obvious but also a management by year interaction. Generally, a high and significant correlation was observed between crude protein content and baking volume (Fig. 1).

## Keywords

Baking volume · crop rotation · grain yield · pre-crop · protein content · *Triticum aestivum*

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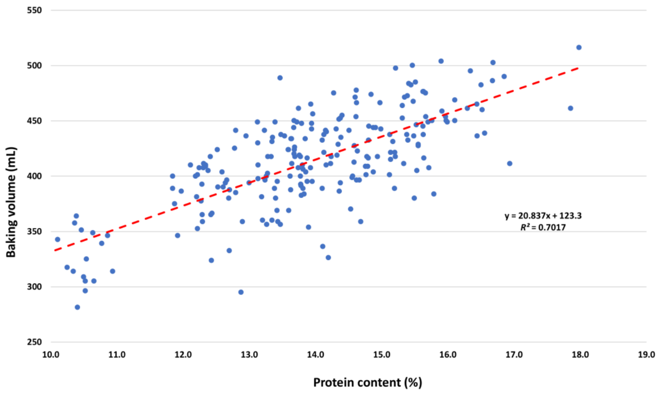

**Figure 1** Relationship between crude protein content and baking volume across all test entries and trials.

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**Table 1** Grain yield and quality traits in the winter wheat trials, 2019-2021.

| | 2019 | | | 2020 | | | 2021 | | |
|---|---|---|---|---|---|---|---|---|---|
| | HI[2] | LI | Org | HI | LI | Org | HI | LI | Org |
| GYLD[1] ($t \cdot ha^{-1}$) | 13.3 | 12.5 | 7.5 | 12.3 | 10.6 | 5.1 | 9.5 | 9.1 | 8.1 |
| PROT (%) | 12.8 | 14.1 | 14.4 | 14.2 | 14.0 | 9.5 | 15.2 | 15.5 | 13.5 |
| GLUT (%) | 30.1 | 33.8 | 32.6 | 38.2 | 35.8 | 19.6 | 44.7 | 44.7 | 35.1 |
| SDS (mL) | 73.1 | 76.1 | 77.1 | 76.6 | 78.2 | 59.1 | 82.5 | 82.2 | 79.9 |
| HLW ($kg \cdot hL^{-1}$) | 80.4 | 80.5 | 80.4 | 81.8 | 81.9 | 79.9 | 74.4 | 74.2 | 75.5 |
| HFN (s) | 374 | 334 | 436 | 379 | 382 | 278 | 262 | 281 | 272 |
| VOLU (mL) | 393 | 392 | 412 | 407 | 411 | 315 | 437 | 440 | 419 |

[1] GYLD, grain yield; PROT, protein content; GLUT, wet gluten content; SDS, SDS sedimentation value; HLW, hectolitre weight; HFN, Hagberg falling number; VOLU, baking volume

[2] HI, Stupice high-input conventional trial; LI, Stupice low-input; Org, Uhrineves organic trial

72. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
22.-24. November 2021, Online Conference, Raumberg-Gumpenstein, Österreich

# Evaluation and selection of durum wheat accessions suitable for organic production

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## Abstract

A durum wheat nursery was tested in Central Italy for three years with the aim to select the most suitable durum accessions for organic production. A total of 72 accessions were evaluated in 2019, the best 27 accessions were tested in two further years, 2020 and 2021. Pre- and post-harvest measurements were recorded during the whole growing season. In parallel, an experiment was performed with 16 accessions at three farms located in three different environments in Central Italy, from inland to seaside.

## Keywords

Agricultural sustainability · ideotype · organic breeding · participatory breeding · selection with farmers · *Triticum durum*

## Introduction

Plants are exposed to various environmental changes such as rainfall distribution, loss of soil fertility, increase of temperatures and decline of biodiversity (Cramer *et al.,* 2018). These factors are also increasing the abiotic and biotic stresses which affect the amount and the quality of the total annual cereals production (Bot & Benites, 2004; Mann & Gleik, 2015; Nam *et al.* 2015). In view of problems such as pollution, global warming and population growth, FAO (2018) registered a rise of product demand up to 50% by 2050. The project objective is to develop a new durum wheat ideotype suitable for organic production which allows less input (such as fertilizer, herbicide, fungicide and insecticide) to prevent pollution, but provide satisfying levels of grain yield and quality.

## Material and methods

The three-year experiment was performed at the experimental farm of Tuscia University located in Viterbo, Italy. In the first year 72 accessions of different origins were evaluated (*i.e*. 30 accessions from Hungary, 25 from Middle-East and 17 from seed companies developed in the Mediterranean area). A randomised block design with three replications was used. The plot size was 6.13 $m^2$ with a sowing density of 122.6 g per plot. The field was of low fertility and was fertilized with 7.7 $t \cdot ha^{-1}$ organic fertilizer (Fertil 12/5) applied before sowing. During the vegetation period, the phenological stage was recorded using the BBCH scale (Lancashire *et al.* 1991), as well as biotic and abiotic stress tolerance, plant height, yield, and grain quality. Based on the first year results the best 27 accessions (*i.e*., 8 accessions from Hungary, 10 from Middle-East and 9 from seed companies) were selected for further trials. The following two seasons a randomised block experiment with three replications, 7.28 $m^2$ plot size and a sowing density of 234.7 g per plot was used. The same evaluations as in the first year were carried out. To maintain genotypes' purity, separate multiplication plots (ear-to-row progenies) were sown each year. Additionally, climate data were recorded (*i.e*., rainfall, minimum and maximum temperature, evapotranspiration). In parallel, a participatory plant breeding experiment was performed with some accessions during the season 2020/2021 at three farmers' fields with different environmental characteristics located in Central Italy. The locations were: (i) Montalto di Castro, close to the sea at 68 m a.s.l., where accessions with reported tolerance to drought and salt stress were sown; (ii) Rieti, a mountain environment at 400 m a.s.l., where accessions from Hungary which are more tolerant towards winter damage and cold stress were sown; (iii) Viterbo, a hilly environment at 300 m a.s.l., where a mix of both types of accessions was sown. All accessions were evaluated under organic conditions in 30 $m^2$ plots with a sowing density chosen by the respective farmer based on the sowing density used in that area. The statistical analysis was carried out with DSAASTAT (Onofri, 2007).

## Results and discussion

ANOVA for the phenological stages revealed significant main effects (*i.e*., genotypes, years) and a significant genotype by year interaction. Multiple mean comparisons identified three major groups of precocity. Early accessions were already in the middle of heading and/or anthesis at the measurement in early April, while medium accessions were at the end of booting and/or heading, and late accessions were at the beginning of booting (Fig. 1). The differences in precocity match with the origin of the accessions; early and medium accessions were developed in the Mediterranean Basin, while the late group included all the Hungarian lines plus one ancient Italian variety. Differences in precocity affected also plant growth in the first period of the season. Late accessions demonstrated a more prostrate growth habit while medium and early accessions showed an erect growth. Precocity was inversely related to soil cover which was higher for the early and medium

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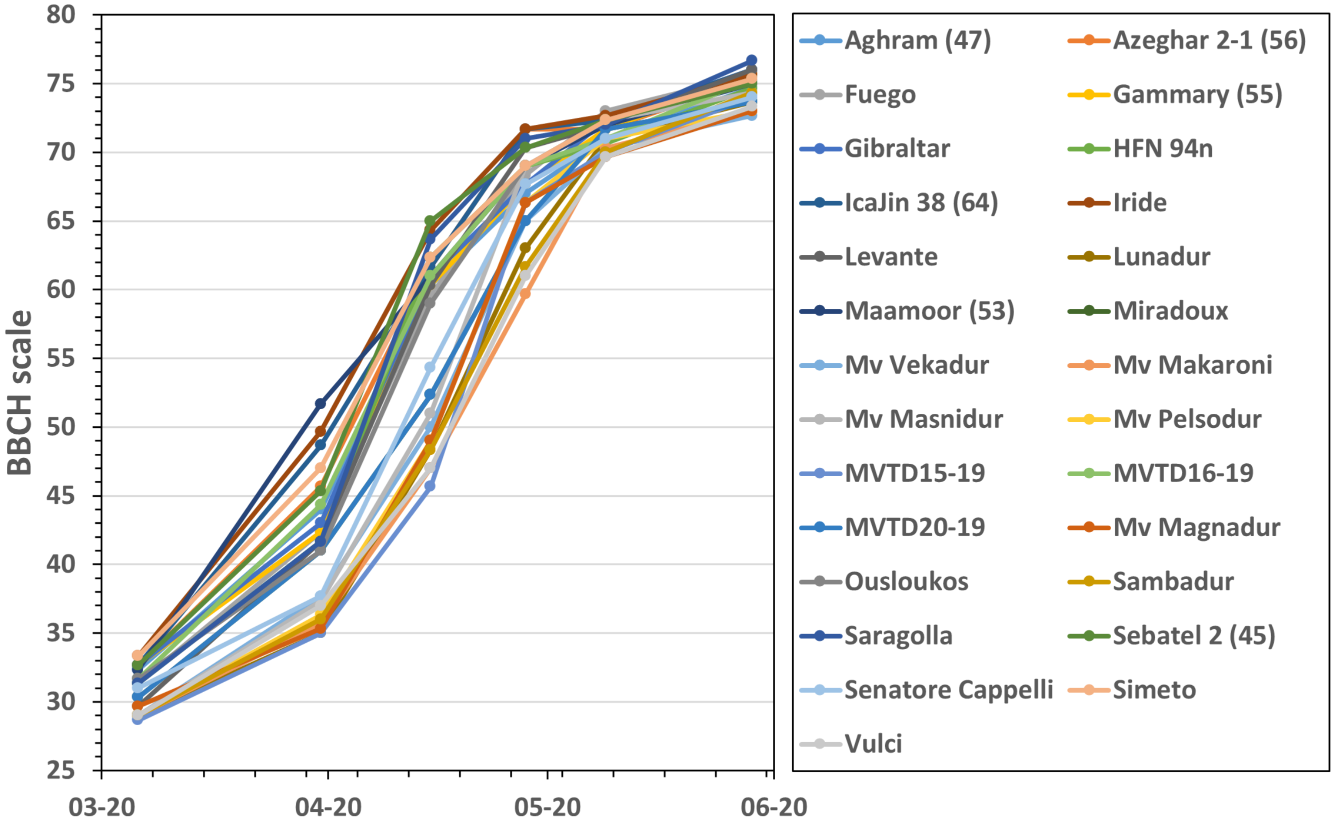

**Figure 1** Phenological development of the ECOBREED durum wheat nursery in Viterbo, Italy. BBCH growth stage is a mean from three years' trials (2019-2021). Date on x-axis is given in the MM-DD format.

accession than the late accessions. Precocity, however, did not affect grain yield and quality, which were uniformly distributed for all accessions with no influence of their origin. This difference in precocity is an important trait which can be used as a strategy for planning a growing season of durum wheat more suitable for each specific area (environment).

The parallel experiment performed at the three farms during 2020/21 was highly affected by adverse environmental conditions, such as excessive rainfall and cold stress. In fact, a drastic drop of temperatures was registered during some nights in spring which led to a severe winter damage particularly in Viterbo (Fig. 2a) where the Hungarian cvs. 'Mv Makaroni' and 'Mv Pelsodur' outyielded

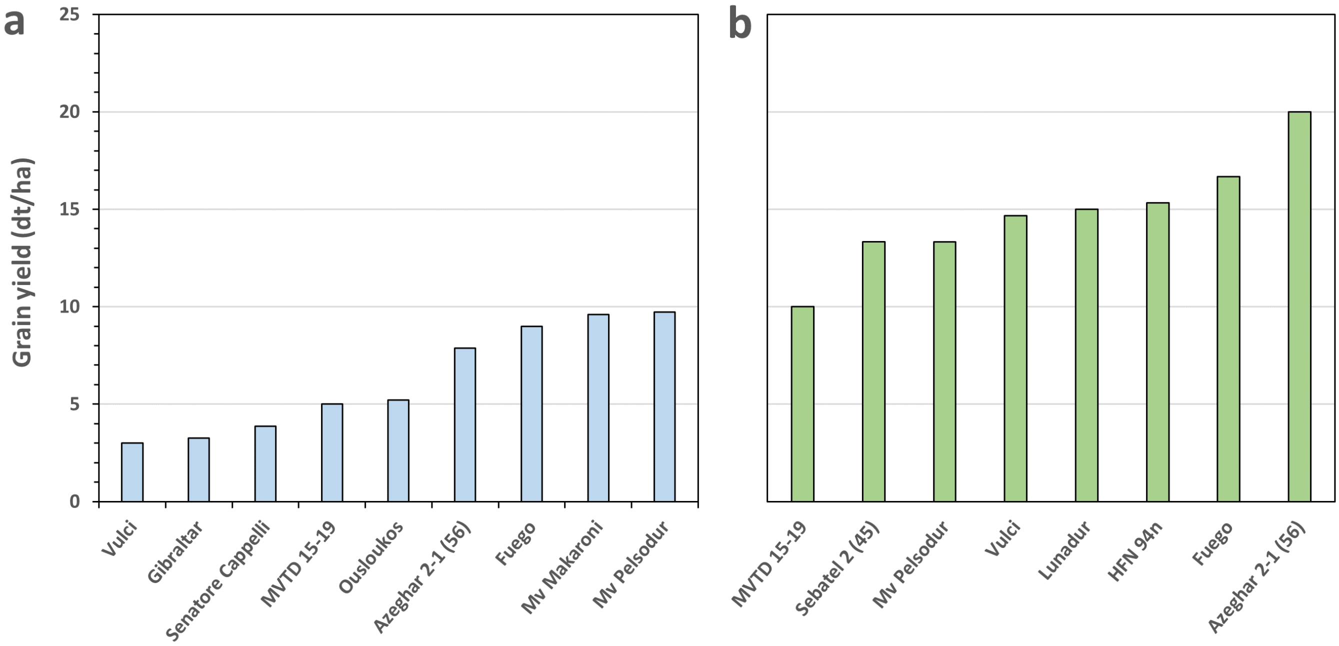

**Figure 2** Grain yield of durum wheat varieties in the farmer participatory trials 2021: **a** Viterbo; **b** Montaldo di Castro

the Mediterranean varieties. In Rieti, the experiment was heavily affected high rainfall and low temperatures. In Montalto di Castro, winter damage was lower resulting in higher grain yields (Fig. 2b). From these trials the involved farmers have evaluated and selected those accessions which they wanted to keep for the next year trial.

## Acknowledgements

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

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# Evaluating salt effects on durum wheat root system using non-invasive phenotyping technique at early plant developmental stages

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## Abstract

Water and nutrition availability for plant roots decreases due to the high soluble salt concentrations in the soil, which negatively affect crop development and productivity. This study was conducted to better understand the relationships between root system traits and tolerance to salt in six contrasting durum wheat genotypes. Results showed that salt effects on durum wheat root and shoot traits was significant, overall negative, but varied between genotypes and over time.

## Keywords

*Triticum durum* · seedling · salinity · root phenotyping

## Introduction

According to FAO (2022) soil data collected in more than 250 000 locations across 118 countries, representing about 85% of global land area, has shown that more than 400 million hectares of topsoil and more than 800 million hectares of subsoil are salt-affected. The soil and water salinity is a harmful abiotic factor that reduces crop productivity, causing the greatest damage for grain production all over the world (Shrivastava & Kumar, 2015). Plant root system plays a critical role in the uptake of water and nutrients, and is also the first organ that senses and responds to salt stress. Root phenotyping can help to determine root architectural traits to be selected in durum wheat for increasing salt-tolerance. In comparison with the aboveground plant part, roots are not easily accessible by non-invasive analyses. However, using non-invasive root phenotyping platforms, such as the GrowScreen-Rhizo (Nagel *et al.*, 2012), allow automatic imaging of roots and shoots of plants grown in soil-filled rhizotrons (Tracy *et al.*, 2020). The aim of this study was to evaluate salt effects on the root traits of six contrasting durum wheat genotypes at early developmental stages, using the GrowScreen-Rhizo platform.

## Material and methods

*Plant material*
Plant material consisted of highly salt tolerant cv. 'Jennah Khetifa', moderately salt tolerant cv. 'Cham 1', which have been analyzed molecularly (Mondini *et al.*, 2011; 2012; 2015), and four lines with contrasting root angles that are also under evaluation in the framework of the ECOBREED Horizon 2020 project.

*Experimental design*
A randomized complete block design was used with 6 replications per genotype and two treatments (control and salt). In order to choose a stress level sufficiently high to cause a visible effect within a few weeks of treatment, but not too strong to kill the plants, two pilot experiments were performed for approximately 3.5 weeks. As a result, a salt treatment of 500 mM NaCl was chosen in the trial. Imaging of roots and shoots was performed seven times at 3, 6, 10, 13, 17, 20 and 24 days after seedlings had been transferred to the rhizotrons of the GrowScreen-Rhizo platform (Fig. 1). In total, eleven traits were examined: (i) total root length, (ii) root length of seminalnodal and (iii) lateral roots, (iv) root system depth and (v) width, (vi) convex hull area, (vii) SPAD, and after harvest, (viii) shoot fresh and (ix) dry weight, (x) root dry weight, and (xi) leaf area.

## Results and discussion

The results showed that the effect of salt stress was significant among the durum wheat genotypes, and over time. For example, significant effects of salt treatment on seminal root length were detected already after 10 days, while for lateral roots the significant effect was visible after 13 days. Total root length under salt treatment showed significant effects only after 17 days. Statistical analysis showed also that root system depth was significantly affected by salt during the whole experiment, while root system width was not affected at all by salinity. Results indicate that the main effect of salt stress is a change in root distribution, *i.e.*, from deep to horizontal growth. In addition, salt stress affects the whole root system by reducing it. The characteristics highlighted in the highly salt-tolerant genotype 'Jennah Khetifa' can be used as alter-

Urbanavičiūtė I, Bongfiglioli L, Nagel KA, Pagnotta MA (2022) Evaluating salt effects on durum wheat root system using non-invasive phenotyping technique at early plant developmental stages. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 72. Jahrestagung 2021, 22-24 November, Raumberg-Gumpenstein, pp 59-61. University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-96-1

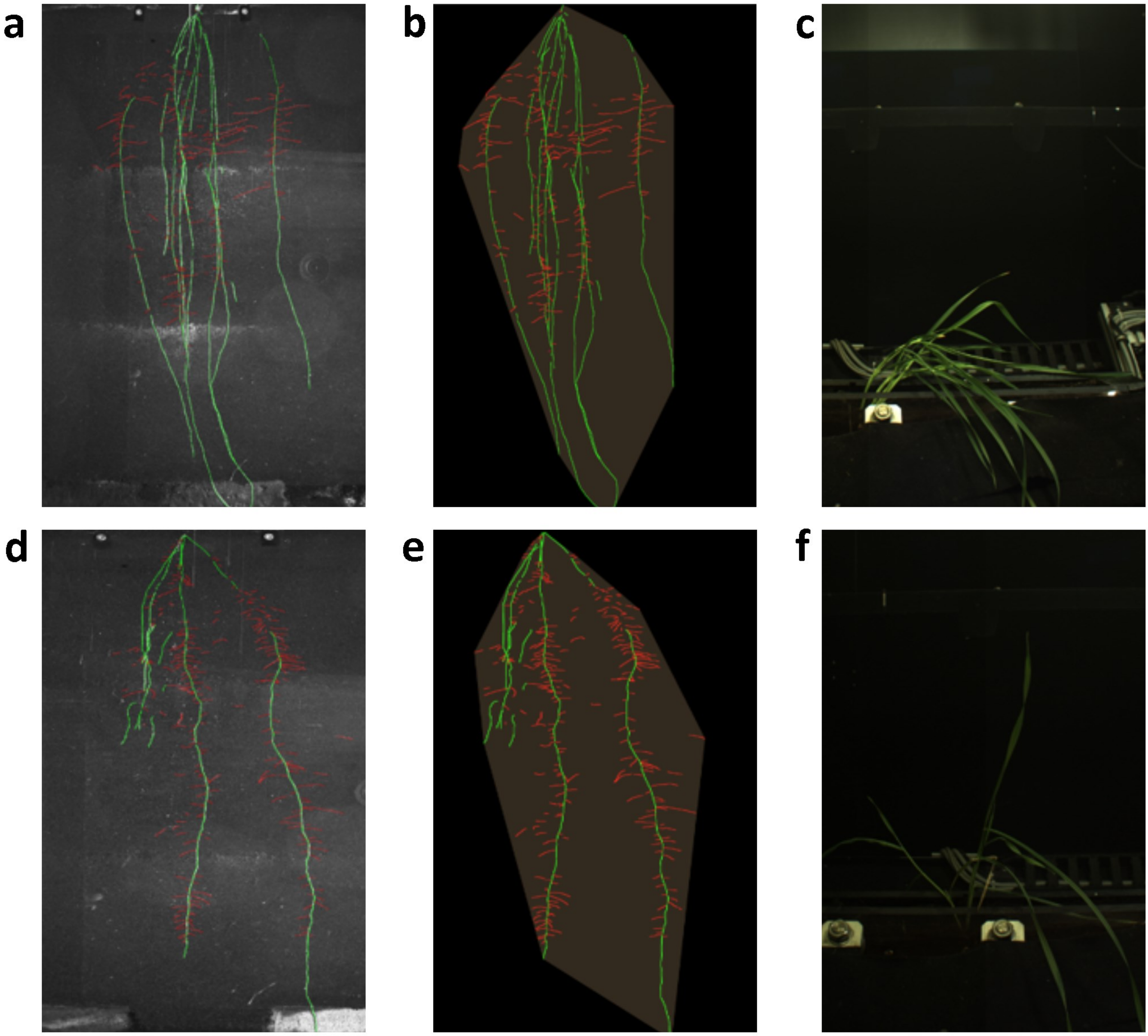

**Figure 1** Highly salt-tolerant durum cv. 'Jennah Khetifa' grown under **a-c** control treatment and **d-f** 500 mM NaCl treatment. **a, d** Root system image taken 24 days after transplantation; main roots are in green and lateral roots in red; **b, e** Convex hull area of the root system; **c, f** Shoot image taken 24 days after transplantation

native parameters for selecting tolerant genotypes. For example, 'Jennah Khetifa' maintained the longest roots under salt conditions during the whole experiment and had fewer negative effects on root system depth compared to control plants (Fig. 1).

Moreover, 'Jennah Khetifa' produced the highest shoot biomass and largest leaf area under both treatments, while 'Vulci' exhibited the lowest values in all shoot traits. Our results showed that among the analysed genotypes 'Sebatel' was most similar to 'Jennah Khetifa' and could, therefore, be used in breeding programs of durum wheat for salinity tolerance.

## Acknowledgements

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# Compatibility screenings of wheat cultivars with arbuscular mycorrhizal fungi: lessons from pot and field experiments

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## Abstract

More than 80% of land plants including crop species such as *Triticum aestivum* form symbioses with arbuscular mycorrhizal fungi (AMF). This type of symbiosis offers benefits to host plants such as increased nutrient status and tolerance to abiotic and biotic stresses. The effects are often highly variable as they depend on the fungal isolate(s), plant species, cultivar and environmental conditions. Also in wheat, genetic variability with regard to the formation of symbiosis has recently been demonstrated in 94 bread wheat genotypes. As AMF provide several benefits to their host plants, AMF compatibility should be an additional feature of interest for organic breeding programs especially with regard to abiotic stress tolerance. However, compatibility tests under standardized greenhouse conditions with single AMF strains might not reflect the field situation with native AMF. Here, we evaluated the AMF compatibility of selected wheat genotypes in a two-step process starting with standardized pot experiments with a single AMF strain and a consecutive field test with native AMF communities.

Two greenhouse pot experiments were established with 20 different genotypes each giving a total of 40 genotypes tested. For AM inoculum production, *Funneliformis mosseae* (BEG12) was proliferated on *Plantago lanceolata* grown in a mixture of sand and expanded clay at a 1:1 (v/v) ratio for several months. The inoculum (root fragments, spores, hyphae, sand and expanded clay) was added at a rate of 2.5% (v) per pot of low nutrient substrate (1000 mL). For each genotype, five pots with AMF and respective control pots were prepared. After six weeks of cultivation, the substrate was removed and the roots were washed under tap water. Roots were stained with ink and vinegar. For each sample 30 root pieces were mounted on a microscope slide and AMF colonization was determined by scoring the frequency of mycorrhiza and the intensity of mycorrhizal colonization. In October 2020, a field experiment in a completely randomized design was established at the experimental farm in Gross Enzersdorf (Austria) to screen preselected wheat genotypes (*i.e.,* 'Aristaro', 'Aurelius', 'Bauermeister', 'Ehogold', 'Mv Kolompos', 'Spontan') for their compatibility with native AMF. Roots of four plants per plot were sampled at a soil depth of 15 cm at the flowering stage and further processed for assessment of AM root colonization as described above.

All selected genotypes were colonized by *F. mosseae* in the greenhouse pot experiments (data not shown). The substrate was low in nutrients to facilitate AM colonization and to reveal genotypes that show a high readiness for colonization under these circumstances. Differences between the genotypes were mainly seen in the mycorrhizal intensities in the root system. The cultivars 'Bauermeister' and 'Spontan' had the highest AM intensities between 22% and 26%, respectively (Fig. 1). The lowest intensity was found in the cultivar 'Ehogold' with 1%. Based on these results, the following wheat genotypes 'Aristaro', 'Aurelius', 'Bauermeister', 'Ehogold', 'Mv Kolompos' and 'Spontan' were selected for the field experiments in order to cover the whole genetic variability of response to AMF colonization in the greenhouse pot experiments. In the field, all genotypes showed similar high levels of colonization (Fig. 1). Only 'Bauermeister' with very high intensities in the greenhouse showed slightly lower values in the field situation. These results confirmed that AMF colonization levels even in very distinct genotypes are dependent on factors such as AMF strains and environmental conditions. Moreover, variation in colonization levels in the field appeared minimal indicating a high ability of the selected genotypes to engage in a symbiotic relationship. For these genotypes it can be concluded that they had not lost their ability to form a symbiosis with AMF during various selection processes. Further analysis of AMF communities in the very same samples will give more insight in the AMF community structure and possible host–genotype colonization preferences. In conclusion, we propose that additional to pot tests with single strain isolates field tests on different sites with natural communities are required to reveal the full colonization potential and to avoid over- or underestimation of AMF compatibility.

Hage-Ahmed K, Lebesmühlbacher J, Baumgartner S, Bilsborrow P, Grausgruber H (2022) Compatibility screenings of wheat cultivars with arbuscular mycorrhizal fungi: lessons from pot and field experiments. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 72. Jahrestagung 2021, 22-24 November, Raumberg-Gumpenstein, pp 63-64. University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-96-1

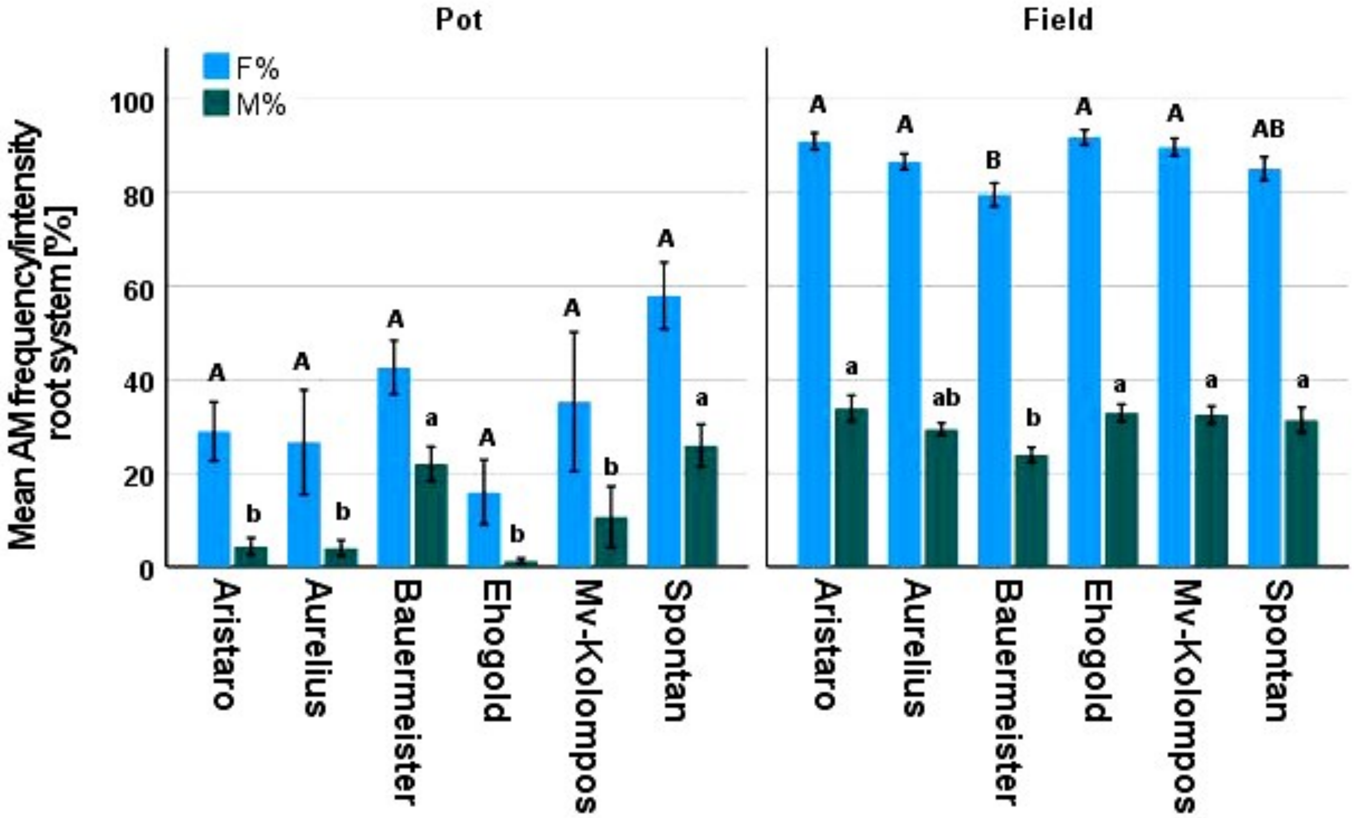

**Figure 1** Arbuscular mycorrhizal frequency (F%) and intensity (M%) in the root system of six different wheat genotypes from pot ($n$ = 5) and field experiments ($n$ = 16). Bars sharing the same letters are not significantly different (mean ± S.E., one-way ANOVA, Student-Newman-Keuls Test, $p < 0.05$).

**Keywords**

AMF colonization · organic farming · plant-microbe interaction · symbiosis · *Triticum aestivum*

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# Evaluation of marker-assisted selection for introgressed exotic common bunt resistance QTL in a back-cross population

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## Abstract

Common bunt is a seed-borne disease of wheat which can lead to major losses in grain yield and quality by replacing kernels with so-called “bunt balls” consisting of fungal teliospores. Owing to their life cycle, the causal agents *Tilletia tritici* and *T. laevis* mainly affect winter wheat. Under organic farming conditions, the highly effective seed dressings with systemic fungicides are prohibited, making resistant cultivars the most efficient way to manage the disease. Host plant resistance against common bunt is considered to be mainly based on gene-for-gene interactions, but also quantitative differences in resistance are common. These could be important to achieve more durable types of bunt resistance, since breakdowns of single resistance genes have already been observed. The projects ECOBREED and DIGIBREED aim to use marker-assisted selection (MAS) to pyramide bunt resistance QTL, and to combine MAS and genomic assisted selection (GAS) into an optimal selection scheme, respectively, for populations with introgressed exotic resistance alleles to facilitate the rapid development of breeding lines with durable resistance against common bunt. The efficiency and accuracy of both selection methods shall be evaluated in field trials to determine the most suitable combination of both strategies.

In the scope of the projects, we developed a back-cross population with 218 lines based on initial crosses between elite cultivars and one out of three resistance donors (*i.e.*, ‘Blizzard’, ‘Bonneville’ - both US cultivars released in 1989 and 1994, respectively; PI 119333 - a Turkish landrace and carrier of resistance gene *Bt12*). $F_1$ plants were back-crossed two times to other elite cultivars or breeding lines to develop lines with a high variation in the elite genetic background. Marker-assisted foreground selection for known common bunt resistance loci and genomics-assisted background selection for agronomically relevant traits was performed on $F_1$ and $F_2$ lines. For MAS, Kompetitive Allele-Specific PCR (KASP) markers for loci on five different chromosomes were applied. If suitable polymorphic markers were available, at least two markers flanking the chromosomal region of the respective resistance locus were used. Common bunt resistance of selected lines was tested in an artificially inoculated field trial at IFA Tulln. Grain samples were inoculated prior to sowing with a spore suspension of bunt teliospores in a solution of methylcellulose in water which was distributed onto the seeds by shaking. The trial was sown as a randomized complete block design in two replications. At the time of ripening, common bunt incidence was assessed by cutting 75 spikes per plot open and checking for the presence of bunted kernels inside. In addition, heading date, plant height, awnedness, plot density and lodging tolerance were assessed.

The process of MAS is already completed and results from the first field trials are available which allow conclusions about the applicability of this method for selecting lines with resistance QTL inherited from the donors. Out of the 218 lines phenotyped for common bunt incidence in 2021, 70 were completely resistant. The panel tested in the field comprised 158 lines which were positively selected and also 60 lines which had a low chance of having inherited the resistance loci according to the allele calls of the applied KASP markers. Polymorphic markers at flanking positions of the QTL region were not available for each locus and line, reducing the probability of accurate selections for some genotype-QTL-combinations. Furthermore, it was known from previous studies that the tested loci confer different levels of resistance against common bunt. These factors explain the comparably high number of lines which were positively selected but still show some degree of infection and also the observation of large differences in incidence levels for individual QTL(-combinations). While lines harboring the resistance loci on chromosome 1B or 7D (*Bt12*) showed very low infection levels and also low variation, those that were selected positively for resistance QTL on chromosomes 1A or 7A showed high mean incidence and large variation in overall infection levels (Fig. 1). Negatively selected lines showed highest average common bunt incidence. Combining the two loci on chromosomes 1A and 1B conferred almost complete resistance while much more variation was observed in lines harboring each locus on its own. These first results indicate, that MAS for quantitative resistance against common bunt in such a population is effective and serves as a suitable tool to reduce the number of lines entering the time- and resource-intensive field testing. Upcoming multi-environment field trials in two locations in Austria as well as one location each in the Czech Republic and the United States will provide additional knowledge about the effectiveness of genomics-assisted background selection in terms of yield and agronomic properties, durability of common bunt resistance in the selected lines and also resistance of these lines to dwarf bunt (*T. controversa*).

Ehn M, Buerstmayr M, Buerstmayr H (2022) Evaluation of marker-assisted selection for introgressed exotic common bunt resistance QTL in a back-cross population. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 72. Jahrestagung 2021, 22-24 November, Raumberg-Gumpenstein, pp 65-66. University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-96-1

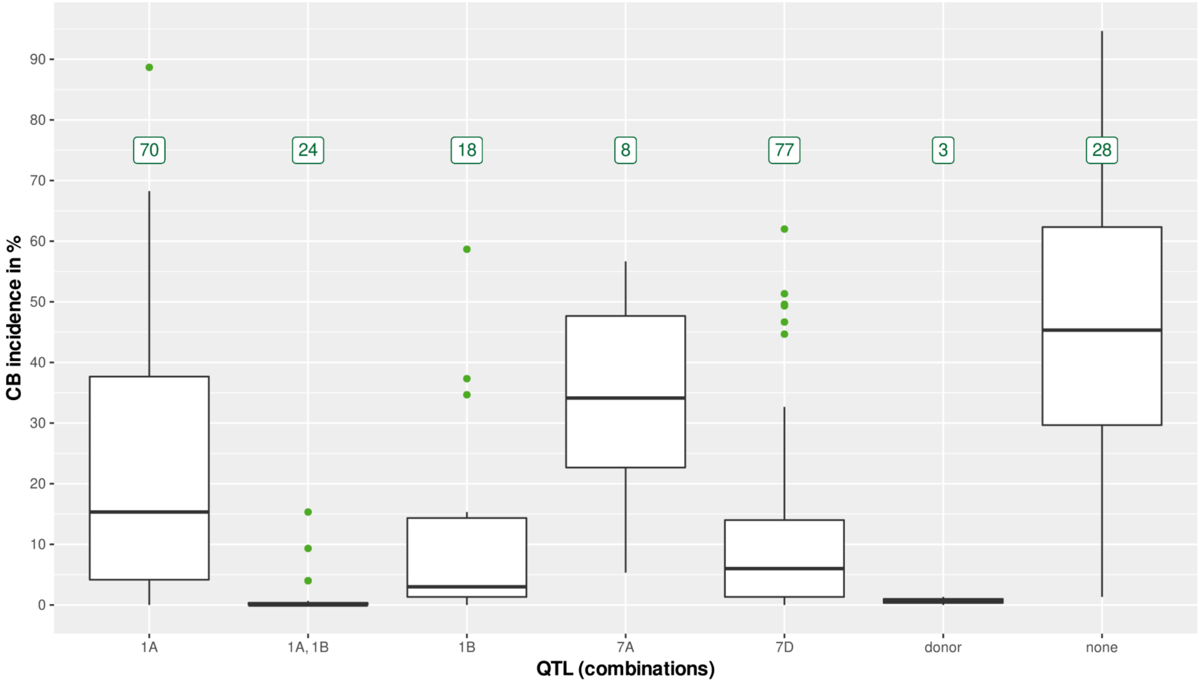

**Figure 1** Boxplots showing common bunt incidence levels in 2021 for lines selected to harbor various resistance QTL or combinations of QTL. The rightmost two boxplots show results for the resistance donors and lines negatively selected for any of the QTL regions. Dots represent outliers. The respective chromosome for each QTL region is denoted on the x-axis. Numbers on top of the boxes indicate the number of lines being assigned to the respective QTL-group.

**Keywords**

Disease resistance · field experiments · MAS · organic farming · *Tilletia caries* · *Tilletia foetida* · *Triticum aestivum*

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# Evaluation of ECOBREED winter wheat germplasm for common bunt resistance

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## Abstract

The Horizon 2020 project ECOBREED aims to (i) test currently available winter wheat varieties for their resistance to common bunt and (ii) develop new organic winter wheat lines resistant to common bunt by marker-assisted selection (MAS). In the present study, 42 multi-parent winter wheat breeding lines were tested in the field after artificial inoculation with common bunt together with 40 varieties and/or resistance sources. The breeding lines were selected after two MAS cycles. Highly resistant breeding lines were identified carrying different resistance QTL combinations. Additionally, moderately resistant lines were identified carrying none of the QTL followed by MAS, indicating that other resistance genes (*i.e.*, from 'Spontan' and 'Genius') were involved in the resistance response of the multi-parent crosses. Several breeding lines and varieties 'Genius', 'Spontan' and 'Unitar' showed a high percentage of spikes with a form of 'partial resistance' with only a few spikelets per spike carrying bunt balls. The results also revealed that artificial testing with different *Tilletia* isolates is necessary due to the presence of genotype×isolate interactions. For example, varieties carrying *Bt10* (*e.g.*, 'Tillstop', 'Tillexus') were resistant against the Czech isolate, but not against the Austrian isolate.

## Keywords

Marker-assisted selection · organic breeding · resistance · seed-borne disease · *Tilletia caries* · *Triticum aestivum*

## Introduction

Common bunt caused by *Tilletia tritici* (Bjerk.) and *T. laevis* (Kühn) is an important seed/soilborne fungal disease in organic wheat growing as effective control by seed treatment with synthetic fungicides is not allowed. Seed treatments authorized for organic farming are available, however, may require investment in new machinery (*e.g.*, brushing machine) and/or affect germination capacity (Borgen, 2004; Wiik, 2021). The production of bunt sori instead of wheat kernels reduce grain yield but also deteriorate wheat end-use quality as the teliospores release trimethylamine, a metabolic compound smelling like rotten fish (Gopal & Sekhon, 1988; Mathre, 2000; Preugschat *et al*., 2014). Therefore, the development of varieties resistant to common bunt is the best and cost-effective method to control the disease in organic wheat production. Hitherto, 16 race specific resistance genes to common bunt have been reported in wheat (Goates & Bockelman, 2012), therof several *Bt*-genes were mapped and molecular markers developed (*e.g*., Laroche *et al*., 2000; Wang *et al*., 2009; Steffan *et al*., 2017; Muellner *et al*., 2020; 2021).

Here we report the results of a first field resistance testing of multi-parent breeding lines developed within the H2020 project ECOBREED and screened for bunt resistance QTL described by Muellner *et al*. (2020; 2021).

## Material and methods

### *Plant material and Marker-Assisted Selection (MAS)*

In total, 82 genotypes consisting of breeding lines, released varieties, notified Organic Heterogeneous Material (OHM), and plant genetic resouces were tested in Tulln, Austria. Additionally, 33 and 9 genotypes of this nursery were tested in Prague, Czech Republic, and Fundulea, Romania, respectively (Table 1; Fig. 2). Most multi-parent breeding lines contain line S5.58 (Blizzard/Rainer), a few additionally lines P101.30 (Bonneville/Rainer) or P106.24 (Rainer/PI119333) (Muellner *et al*., 2020; 2021). $F_1$'s of initial crosses with a resistance donor were back-crossed to another elite variety and hybrids thereof were screened by KASP markers for the heterozygous presence of resistance QTL described by Muellner *et al*. (2020; 2021). Selected plants were further crossed between each other and the hybrids thereof were screened in another MAS cycle for the homozygous presence of resistance QTL. Selected plants were self-pollinated and multiplied for field screening.

### *Artificial inoculation*

Wheat seeds were inoculated with common bunt teliospores before sowing. In Tulln, the seeds were inoculated with an isolate sourced from a susceptible 'Tilliko' stand. In Prague, isolate RUKR, a mixture of local common bunt strains, was used. Similarly, in Fundulea an isolate mixture sourced from six sites was utilized.

Pfatrisch K, Sternbauer M, Dumalasova V, Marinciu C, Grausgruber H (2022) Evaluation of ECOBREED winter wheat germplasm for common bunt resistance. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 72. Jahrestagung 2021, 22-24 November, Raumberg-Gumpenstein, pp 67-71. University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-96-1

*Observations*

At ripening (*i.e.*, soft to hard dough stage; BBCH85 - BBCH87), the percentage of bunted spikes was determined by cutting the spikes of the inoculated rows with secateurs (Fig. 1). Additionally, in Tulln 50 spikes per plot were harvested at maturity which were further analysed in the lab for the percentage of infected grains (bunt sori). In this process, also the percentage of partially infected spikes was evaluated.

## Results and discussion

### *Genotype by isolate interaction*

As resistance to common bunt is race specific (gene-for-gene interaction), the wheat-bunt interaction is characterized by genetic specificity (Hoffman, 1982). In the present study, the Austrian isolate was more aggressive, followed by the Czech and Romanian isolate, as demonstrated by the varieties which were grown on two and three locations and inoculated with local isolates of common bunt. For example, 'Ursita' showed 36.7% infected spikes in Tulln, however, only 10.4% and 2% in Prague and Fundulea, respectively (Fig. 2). 'Tilliko', source of the Austrian isolate, had 24% infected spikes in Tulln, but was resistant in Prague (Fig. 3a). A similar interaction was observed for 'Graziaro', 'Ursita' and breeding line BTX561-7K. 'IS Laudis', 'PS Dobromila' and 'Unitar' were grouped comparatively in Austria and Romania, whereas 'Glosa', 'Mv Karej', and 'Mv Kolompos' were more susceptible in the Austrian experiment. Noteworthy is also the reaction of varieties carrying the *Bt10* resistance gene (*i.e.*, 'Tillexus' and 'Tillstop'). This resistance gene was still effective against the RUKR-isolate (Fig. 3a), but not against the Austrian Tilliko-isolate which confirms earlier observations (Oberforster & Plank, 2021).

**Figure 1** Double-row test plots and cut infected spike in Tulln 2021. In total 75 spikes per row were evaluated (*i.e.*, 50 on the field, 25 in the lab) resulting in 150 spikes per entry.

### *Partially infected spikes*

Generally, a high correlation (*r*=0.93) was observed between the percentage of infected spikes and the percentage of bunt sori (Fig. 3b). However, a few genotypes (*e.g.*, 'Unitar', 'Spontan', 'Genius' and breeding lines including the latter two varieties) showed a

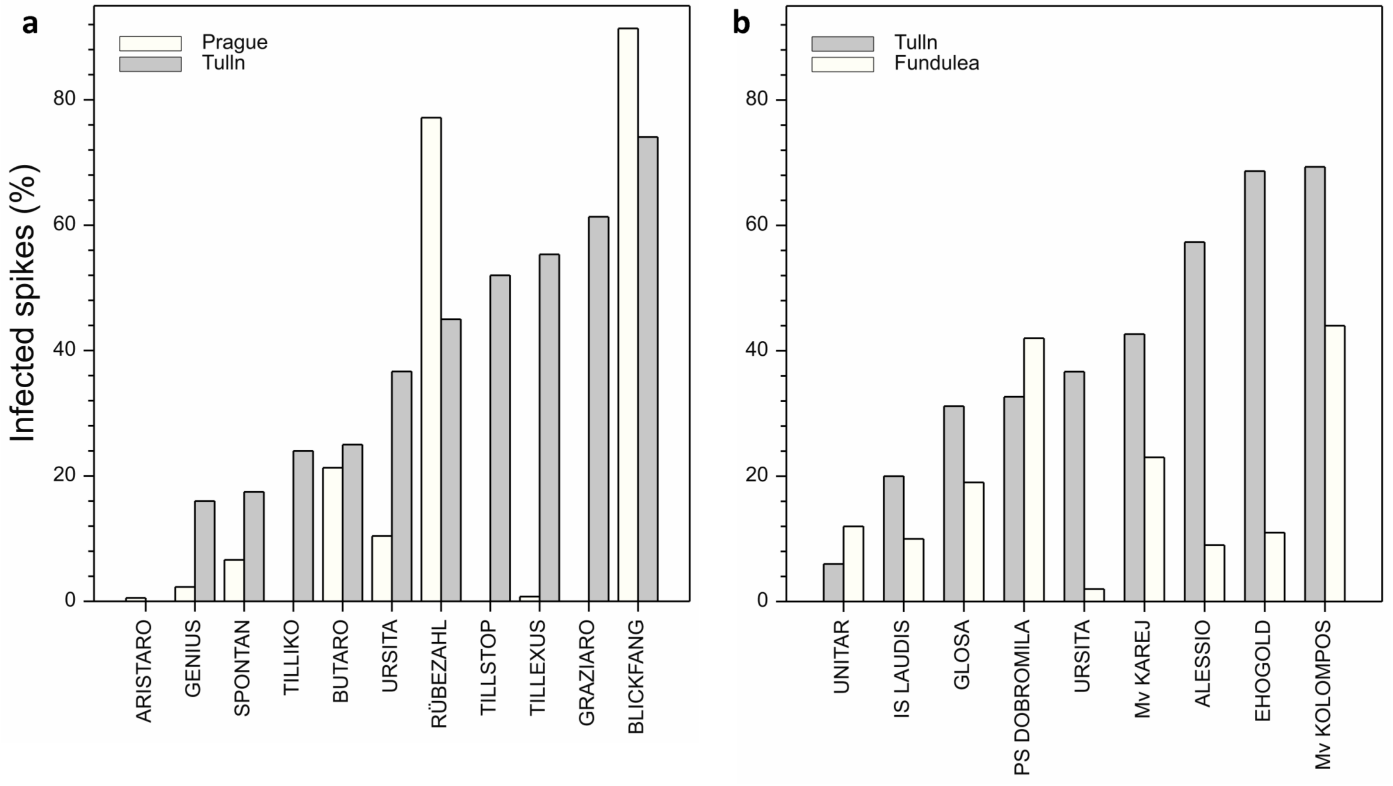

**Figure 2** Susceptibility to common bunt of selected winter wheat varieties from the ECOBREED diversity panel: **a** Percentage of infected spikes in the experiments in Tulln and Prague; and **b** in Tulln and Fundulea. 'Ursita' was included in all three test sites.

**Table 1** Susceptibility of winter wheat to common bunt in Tulln 2021 (CB%, percentage of infected spikes of 150 evaluated spikes; CBS%, percentage of bunt sori of the total number of grains of 50 evaluated spikes) and their resistance QTL

| Genotype | QTL[1] | CB% | CBS% | Genotype | QTL | CB% | CBS% |
|---|---|---|---|---|---|---|---|
| BTX516-6K | 1B, 7D | 0.0 | 0.0 | Heerup | | 21.3 | 20.0 |
| BTX521-1G | 1B, 7D | 0.0 | 0.0 | Creator | | 22.0 | 19.2 |
| BTX559-4 | 1A?, 1B | 0.0 | 0.0 | Tilliko | | 24.0 | 32.4 |
| Aristaro | | 0.0 | 0.0 | BTX522-N0543K | | 24.7 | 20.0 |
| UI SRG | | 0.0 | 0.0 | Butaro | | 25.0 | 12.2 |
| BTX522-4G | 1A, 7D | 0.7 | 0.0 | BTX527-N0539bK | | 26.7 | 19.4 |
| BTX559-14G | 1A?, 1B?, 7D | 0.7 | 0.0 | BTX517-N0532aG | | 28.7 | 13.1 |
| Deloris | | 1.0 | 0.0 | BTX516-N0547K | | 30.7 | 14.7 |
| BTX518-8G | 1B, 7D | 1.3 | 0.1 | BTX528-4G | 1B? | 30.7 | 27.8 |
| 702-1102C | | 2.0 | 0.0 | Glosa | | 31.2 | 15.6 |
| BTX534-5K | 7A, 7D | 2.0 | 0.9 | PS Dobromila | | 32.7 | 29.9 |
| BTX516-10G | 1A, 1B | 2.7 | 3.2 | BTX561-7K | 1B? | 33.2 | 8.4 |
| BTX517-N0532aK | | 3.3 | 2.3 | BTX522-N0543G | | 36.2 | 20.2 |
| BTX516-11G | 1A, 1B | 5.3 | 2.5 | Ursita | | 36.7 | 13.2 |
| BTX521-N0533aG | | 5.6 | 5.1 | Pizza | | 38.7 | 32.8 |
| BTX517-11K | 1A, 1B | 6.0 | 1.4 | Viki | | 39.0 | 47.2 |
| BTX559-9G | 1B?, 7A, 7D | 6.0 | 4.3 | BTX561-N0540aK | | 40.7 | 36.6 |
| Unitar | | 6.0 | 0.1 | Wendelin | | 41.3 | 56.7 |
| BTX521-11K | 1A?, 1B | 7.3 | 4.6 | Mv Kárej | | 42.7 | 26.9 |
| BTX521-14K | 1A, 7D | 7.3 | 2.9 | Rübezahl | | 45.0 | 31.8 |
| BTX516-5K | 1A, 7D | 7.4 | 2.8 | Skagit 1209 | | 46.2 | 29.2 |
| BTX516-N0547G | | 8.0 | 7.7 | Red Russian | | 49.3 | 57.0 |
| BTX517-10 | 1A | 8.7 | 9.1 | Royal | | 50.7 | 55.2 |
| BTX518-N0533bK | | 9.3 | 5.2 | Tillstop | | 52.0 | 70.1 |
| BTX527-5 | 1A?, 1B | 10.0 | 11.9 | BTX534-N0548bK | | 55.3 | 34.9 |
| SEC261-05 | | 10.0 | 3.1 | Tillexus | | 55.3 | 76.8 |
| BTX522-6GUF | 1A, 1B | 11.3 | 2.6 | Alessio | | 57.3 | 72.0 |
| BTX527-N0539aG | | 12.0 | 4.8 | Brandex | | 58.0 | 42.8 |
| BTX534-3 | 1A, 1B? | 12.0 | 8.4 | Sheriff | | 58.0 | 83.0 |
| BTX528-11GK | 1A, 1B? | 13.2 | 2.3 | Skagit 1109 | | 60.0 | 60.8 |
| BTX561-N0540aG | | 13.3 | 6.4 | Graziaro | | 61.3 | 74.8 |
| BTX561-8G | 1B? | 15.3 | 7.4 | Wital | | 62.0 | 51.9 |
| BTX521-3K | 1A?, 1B?, 7D | 15.9 | 15.8 | Tengri | | 63.3 | 59.0 |
| BTX518-11G | 1A, 7D | 16.0 | 5.1 | Tobias | | 64.0 | 36.3 |
| BTX534-7K | 1B?, 7D | 16.0 | 7.6 | BTX518-3K | 1A, 7D | 66.0 | 52.8 |
| Genius | | 16.0 | 5.3 | Ehogold | | 68.7 | 67.7 |
| BTX528-N0544G | | 17.3 | 6.2 | Mv Kolompos | | 69.3 | 66.2 |
| Spontan | | 17.5 | 4.4 | Purino | | 71.3 | 60.5 |
| BTX521-N0533bK | | 18.7 | 10.0 | Blickfang | | 74.1 | 76.8 |
| IS Laudis | | 20.0 | 17.1 | Wiwa | | 77.9 | 88.6 |
| BTX527-12 | 1A?, 1B | 20.7 | 14.8 | Annie | | 81.3 | 81.6 |

[1] present QTL according to two cycles of MAS; donor of resistance QTL was in all BTX lines cv. ‘Blizzard’ with the exception of BTX534-3 and BTX534-7K where the resistance QTL on chromosomes 1A and 1B could have also been derived from cv. ‘Bonneville’.

high percentage of partially infected spikes, and therefore a lower percentage of bunt sori compared to the percentage of infected spikes (Table 1). Partially infected wheat grains were reported by Gassner (1938). Hansen (1958) explained partially infected grains by the fact that the ovule is fertilized before the fungus’ invasion. The transformation of the integuments to the testa forms an effective barrier against the further spread of the pathogen. In the present study, healthy-looking grains of partially infected spikes were not checked in detail, however, it was observed that the bunt sori were located more or less randomly within the spike which would disagree with a resistance mechanism against the systematic invasion of the apex.

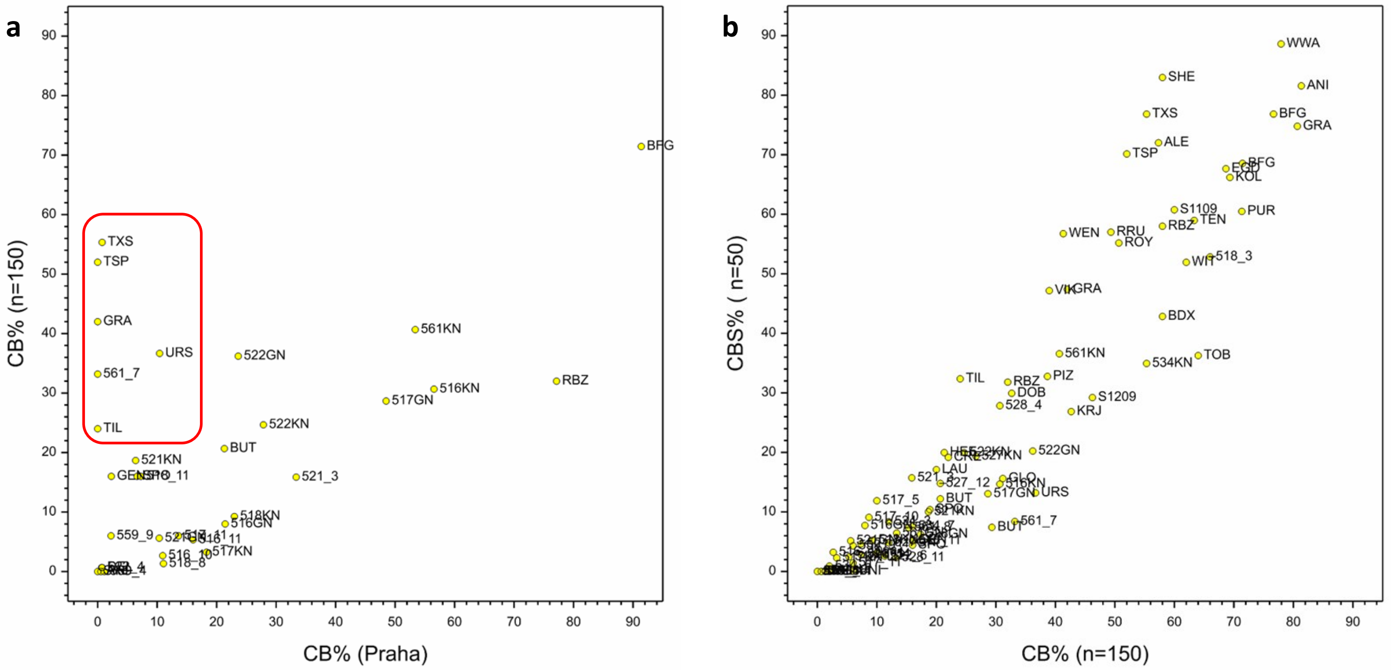

**Figure 3** Common bunt in ECOBREED winter wheat germplasm: **a** Percentage of common bunt infected spikes (CB%) in Tulln 2021 vs. Prague (Praha) 2021 (GRA, Graziaro; TIL, Tilliko; TSP, Tillstop; TXS, Tillexus; URS, Ursita); **b** Percentage of infected spikes (CB%) vs. percentage of bunt sori (CBS%) in Tulln 2021.

### *Resistance QTL*

From Table 1 it is obvious that breeding lines with one or more resistance QTL were more resistant to common bunt than breeding lines without any of the QTL followed by MAS. With respect to the individual QTL it has to be considered that *QBt-ifa.7DS* from ‘Blizzard’ was associated only with dwarf bunt and not common bunt in the study of Muellner *et al*. (2021). Hence, it is assumed that the presence of this QTL had no effect on the performance of the breeding lines against common bunt. Some lines without any resistance QTL (*e.g*., BTX517-N0532aK and BTX521-N0533aG) showed a low susceptibility to common bunt. The resistance of these lines is probably the result of additive effects of involved parents such as ‘Genius’ and ‘Spontan’. ‘Genius’ is also a parent of ‘Tillsano’ which showed a low to medium susceptibility against various common bunt races in Austria (AGES, 2021; Ritzer *et al*., 2022). On the other hand, also Muellner *et al*. (2021) observed some lines of both the mapping and validation populations with low bunt incidence despite having none of the three resistance QTL from the donors ‘Blizzard’ or ‘Bonneville’ (*i.e*., *QBt.ifa-1AL*, *QBt.ifa-1B*S, *QBt.ifa-7AL*). It is noteworthy that *QBt.ifa-1AL* and *QBt.ifa-1B*S, as well as *QBt-ifa.7DS* were detected in many breeding lines, whereas *QBt.ifa-7AL* is present in only two lines. PI 119333, donor of *Bt12*, was involved in 4 BTX-lines, however, the respective QTL *Qbt.ifa-7D/Bt12* (Muellner *et al*., 2020) was not detected in any of these lines. As discussed by Muellner *et al*. (2021) it remains unclear whether *Qbt.ifa-7D/Bt12*, *QBt-ifa.7DS* and/or *Q.DB.ui-7DS* (Chen *et al.,* 2016) represent the same resistance allele.

Like in the study of Muellner *et al*. (2021), lines with only one common bunt QTL were as resistant as lines with two. Hence, epistatic interactions seem to be substantial for common bunt resistance and were confirmed by various other studies (Knox *et al*., 2013; Chen *et al*., 2016; Singh *et al*., 2016). As also outlined by Muellner *et al*. (2021), none of the applied markers is diagnostic for a bunt resistance QTL, but only linked to the QTL. Their value might vanish by ongoing recombination. This might explain the high susceptibility of BTX518-3K despite that *QBt.ifa-1AL* was detected in this line.

## Conclusions

The first screening for common bunt resistance revealed that the applied KASP markers allowed a targeted introgression of resistance QTL from US varieties ‘Blizzard’ and ‘Bonneville’, known for their high and durable resistance, into European winter wheat germplasm. Due to the complex pedigree of the tested lines, additional resistance alleles might be present which could not be followed due to the unavailability of molecular markers. Some genotypes showed only partially infected spikes resulting in a trade-off between bunt incidence (*i.e*., percentage of infected spikes) and percentage of bunt sori in the grain yield. This resistance response needs further confirmation and investigation.

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72. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
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# Research on common bunt of wheat at the Crop Research Institute within the ECOBREED project

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## Abstract

Common bunt caused by *Tilletia caries* (DC.) Tul. & C.Tul. and *T. laevis* J.G. Kühn and dwarf bunt caused by *T. controversa* J.G. Kühn can cause yield losses that may exceed 80%. Bunt diseases threaten farming systems whenever the routine use of chemical seed treatment is not possible. Organic wheat production therefore requires alternative means of common bunt and dwarf bunt control. Breeding for bunt resistance offers an efficient and sustainable plant protection strategy particularly for organic production systems. The aim of this study was to identify bunt resistance in winter wheat varieties and breeding lines.

In common bunt tests, seed was inoculated with common bunt teliospores before sowing. Inoculation was performed by shaking 250 seeds with 0.1 g of teliospores in Erlenmayer flasks for 1–2 min. Inoculations and sowing in 1 m long rows, 0.2 m apart were carried out in October. Dwarf bunt tests were sown in seedbeds in late October. Dry dwarf bunt teliospores were evenly spread on the soil surface shortly after sowing. Soil inoculation was carried out with the doses of 2 g of teliospores per 1 $m^2$. In the absence of snow cover the plots were covered with white nonwoven fabric during the winter months in order to improve conditions for infection. The total amount of spikes and total amount of infected spikes per replicate was counted in July. The reaction to bunt was expressed as the percentage of bunted spikes out of the total number of spikes in the row. An infection incidence >10% indicates virulence.

Inoculum ´RUKR´ used in the field trials at the Crop Research Institute in Prague-Ruzyně consists of a mixture of strains of common bunt of local provenience. It was tested for three years (2019-2021) on a the standard set of wheat differential lines possessing *Bt0* to *Bt13,* and *Btp* resistance genes. It showed a virulent reaction to the genes *Bt1* (PI 554101), *Bt2* (PI 554097), *Bt3* (CI 6703), *Bt4* (PI 11610), *Bt5* (CI 11458), *Bt6* (CI 10061) and *Bt7* (PI 554100). The mean bunt incidence (2019-2021) was 47.4% on *Bt1*, 16% on *Bt2*, 16.6% on *Bt3*, 53.4% on *Bt4*, 43.3% on *Bt5*, 12.3% on *Bt6* and 32.4% on *Bt7*. A resistance response was observed in the differential lines carrying *Bt8* (PI 554120), *Bt9* (PI 554099), *Bt10* (PI 554118), *Bt11* (PI 554119), *Bt12* (PI 119333), *Bt13* (PI 181463) and *Btp* (PI 173437).

In 2021, 37 varieties and breeding lines from the ECOBREED trials were tested in Prague, Czech Republic, and in Tulln, Austria. A comparison of the results from both sites shows that some varieties listed in the CPVO (Community Plant Variety Office) variety database carry bunt resistance genes: ´Aristaro´ showed a common bunt incidence of 0.5% and 0% in Prague and Tulln, respectively; ´Genius´ 2.3% and 9%, and ´Spontan´ 6.6% and 9.5%. Furthermore, ´UI SRG´ and ´Deloris´ (*Bt3, Bt9, Bt10*) derived from breeding programs in the Pacific Northwest region of the United States and three ECOBREED breeding lines were resistant in both localities. Four varieties had resistance genes effective against the inoculum used in Prague, but were susceptible in Tulln: ´Graziaro´ with 0% in Prague but 59% in Tulln, ´Tilliko´ (*BtZ)* with 0% and 17%, ´Tillexus´ (*Bt10*) with 2.3% and 45%, and ´Tillstop´ (*Bt10*) with 0% and 46%.

Some varieties have been tested for common bunt in Prague for many years and have shown a resistant reaction in several years. Among them were the already mentioned cvs. ´Aristaro´, ´Genius´, ´Graziaro´, and ´Tilliko´ with resistance in the experiments 2019-2021, cvs. ´Deloris´, ´Tillexus´, ´Tillstop´ and ´UI SRG´ with resistance in the experiments 2020-2021. Also the US cvs. ´Blizzard´, carrying QTL for bunt resistance on chromosomes 1A, 1B, 7A and 7D, and ´Bonneville´ carrying resistance QTL on chromosomes 1A and 1B were resistant in the experiments performed 2020-2021.

In 2021, 35 genotypes were also tested for dwarf bunt. These genotypes were assumed to carry genes for bunt resistance based on our previous experiments with common bunt. The dwarf bunt incidence was relatively low with 3.6% in the susceptible control ´Heines VII´. ´Tilliko´, resistant in the 2021 common bunt trial in Prague but susceptible in Tulln, had 6.4% of dwarf bunt, which is more than the susceptible control. There was no dwarf bunt incidence in cvs. ´Deloris´, ´UI SRG´ and ´Tillexus´. Also cvs. ´Cardon´, ´Crest´, ´Franklin´, ´Hansel´, ´Lewjain´, ´Manning´, ´Meridian´, ´Philaro´, ´Promontory´, ´Sprague´, ´Stava´, ´SW Magnifik´, ´Ute´, ´Wasatch´ and ´Winridge´ were free of dwarf bunt in the 2021 trial.

Dumalasová V, Grausgruber H (2022) Research on common bunt of wheat at the Crop Research Institute within the ECOBREED project. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 72. Jahrestagung 2021, 22-24 November, Raumberg-Gumpenstein, pp 73-74. University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-96-1

The results with dwarf bunt from previous years revealed that ´Blizzard´ (tested 2013-2016) was resistant; 0.1% dwarf bunt incidence was recorded for ´Bonneville´ (tested 2013-2014). Maximum dwarf bunt incidence of ´Aristaro´ was 0.6% in 2019. ´Butaro´ had 4.7% in 2020, ´Genius´ 5.7% in 2017, and ´Graziaro´ 4.2% in 2020, which could mean a susceptible reaction under conditions more favorable for the development of dwarf bunt.

Resistance to common bunt both in Prague and in Tulln and at the same time resistance to dwarf bunt have so far been only recorded for US cvs. ´Blizzard´, ´UI SRG´ and ´Deloris´. The ECOBREED project seeks to obtain varieties that are resistant to bunt and suitable for European organic growing conditions. It combines marker- and genomics-assisted selection to lead breeding programs towards a successful selection strategy.

**Keywords**

Disease resistance · *Tilletia* spp. · wheat breeding

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# Comparison of pathogenicity of Austrian isolates of *Tilletia caries* on common wheat (*Triticum aestivum*)

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## Abstract

Common bunt caused by *Tilletia caries* and *T. foetida* is a severe disease of common wheat (*Triticum aestivum*) worldwide. Common bunt is especially problematic in organic agriculture because no chemical seed dressing is allowed. Therefore, the exploitation of resistant genetic resources is essential. The virulence patterns of common bunt collected in different regions can differ. Hence, it is crucial to develop regionally adapted cultivars. Our study tested eight common bunt isolates on 40 wheat genotypes, including the differential set (resistance genes *Bt1* to *Bt13, and BtP*). Significant differences among isolates were found. Some differential lines showed high resistance among all isolates: *Bt1, Bt5, Bt6, Bt11, Bt12* and *BtP* seem to be resistant under Austrian conditions. Future breeding programs should incorporate these resistance genes into their lines to provide sufficient disease suppression.

## Keywords

Aggressiveness of isolates · common bunt · differential set · organic wheat breeding · resistance breeding

## Introduction

Common bunt of wheat, caused by both *Tilletia caries* (D.C.) Tul. (syn. *T. tritici*) and *T. foetida* (Wallr.) Liro (syn. *T. laevis*) is a soilborne disease with a high potential for reproduction. Instead of regular grain filling, bunt balls filled with teliospores are produced, which leads to yield and quality losses. The teliospores contain trimethylamine, which causes an unpleasant fish-like smell. Especially in organic agriculture, bunt infections may cause severe problems because chemical seed dressings are unavailable (Matanguihan *et al.,* 2011; Goates, 2012; AGES, 2021). Resistant cultivars are the most efficient way to control the disease. Unfortunately, only a few cultivars resistant to common bunt are available in Austria (AGES, 2021). During the last 15 years, an increase in common bunt incidence has been observed in Austria. Additionally, there is evidence that more aggressive races of common bunt can overcome current resistance sources (AGES, 2021). The study aimed to test different Austrian isolates for their virulence patterns.

## Material and methods

In this study, eight common bunt isolates collected in various regions of Austria were tested on a set of 40 wheat cultivars. Two isolates were collected in field trials of IFA-Tulln, whereas the other six were collected by AGES at different locations in Austria. The test panel included the common bunt differential set (carriers of the resistance genes *Bt1* to *Bt13,* and *BtP*), exotic resistance donors, new breeding lines, and registered cultivars. Each genotype was artificially inoculated with each isolate. Inoculated seeds were sown in November 2020 with two replications per treatment. From the end of May to mid-July 2021, plant traits like ear emergence, flowering time, presence of awns and plant height were scored. Common bunt incidence was recorded by assessing 150 spikes per plot as healthy or diseased by cutting them open and checking for the presence of bunt balls. Data analysis included ANOVA, calculation BLUEs and isolate mean comparisons using Tukey's posthoc test, as well as the calculation of heritability and correlations between traits.

## Results and discussion

Statistically significant differences in common bunt incidence were found between both genotypes and isolates ($p < 0.001$). Mean common bunt incidence across all 40 cultivars was 10.4%. Loosdorf was the most aggressive isolate (12.5%), followed by IFA aggressive (12.1%) and Gerhaus (11.6%). Harmannsdorf (8.3%) and IFA housekeeping (8.3%) were the least virulent isolates in our experiment (Fig. 1). Thening (11.1%), Sitzendorf (10%) and Hinzenbach (9.7%) showed intermediate incidence. Tukey HSD test revealed significant differences between the aggressive isolates Loosdorf, IFA aggressive and Gerhaus, and the low aggressive isolates Harmannsdorf and IFA housekeeping Mix. Based on their similarity in virulence patterns, the eight isolates can be grouped

Ritzer E, Ehn M, Oberforster M, Buerstmayr H (2022) Comparison of pathogenicity of Austrian isolates of *Tilletia caries* on common wheat (*Triticum aestivum*). In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 72. Jahrestagung 2021, 22-24 November, Raumberg-Gumpenstein, pp 75-76. University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-96-1

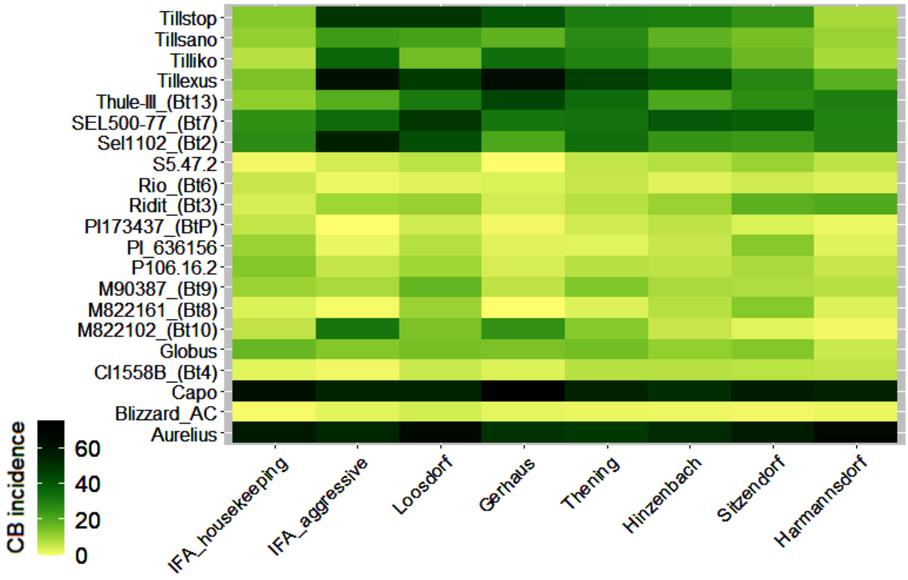

**Figure 1** Heatmap showing the common bunt incidence (%) for susceptible lines (>5% CB incidence; y-axis) tested with eight different isolates (x-axis). Common bunt incidence per isolate is displayed with different color intensities.

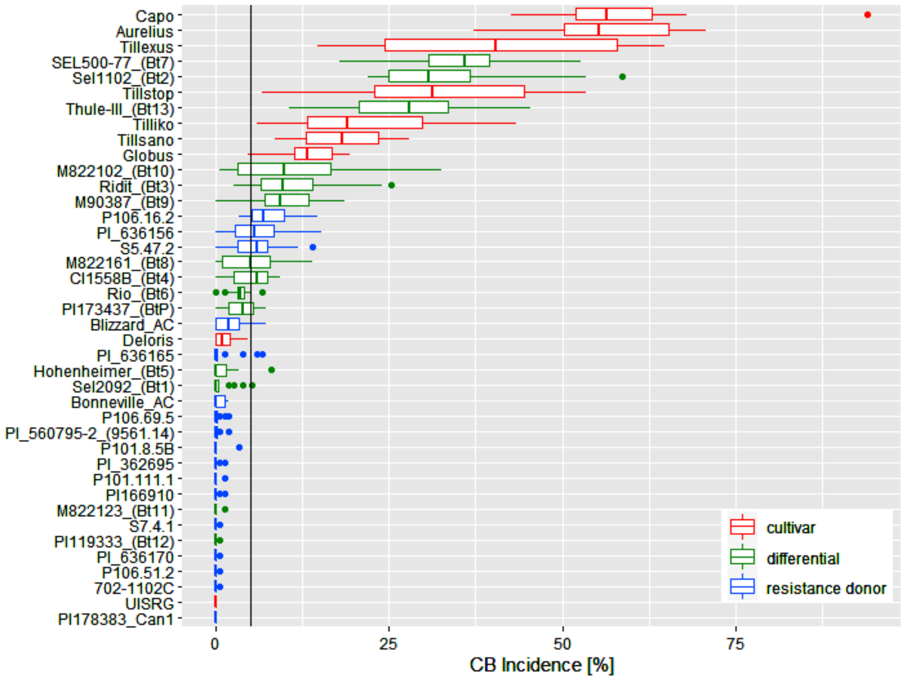

**Figure 2** Variation in common bunt (CB) incidence of tested wheat germplasm. Genotypes are ordered by their mean CB incidence across all eight isolates; the black vertical line indicates a threshold of 5% CB incidence.

into three groups: (i) IFA housekeeping and Harmannsdorf; (ii) Hinzenbach, Thening and Loosdorf; (iii) IFA aggressive and Gerhaus (Fig. 1). The differential lines for *Bt1*, *Bt5*, *Bt6*, *Bt11*, *Bt12*, *BtP* and 13 other genotypes (mostly belonging to the group of resistance donors) showed high or complete resistance (<5% infected ears) against all eight Austrian isolates (Fig. 2). Therefore, future breeding efforts should combine several of these resistance sources. On the other hand, cultivars like Tillstop (31%), Tillsano (18.6%), Tilliko (21.6%) and Tillexus (35.8%), which were considered resistant (AGES 2021), showed moderate susceptibility in the field trial. Therefore, developing new cultivars resistant to common bunt is of great importance, especially for organic agriculture.

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# Enhanced exudation of BOA, HMBOA, HBOA and DIBOA by wheat seedlings in proximity to common purslane (*Portulaca oleracea*) and annual ryegrass (*Lolium rigidum*)

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## Abstract

Allelochemicals are essential for plants to interact with their biotic environment, attract pollinators or seed dispersers, defend against natural enemies and suppress potential competitors. Wheat is an important allelopathic crop that has the ability to produce and release allelopathic metabolites into the environment and can interfere with the growth and development of neighboring plants. The allelopathic potential of wheat straw, root exudates and surface mulch has been documented. Major phytotoxins quantified from wheat include hydroxamic acids, polyphenols and flavonoids. Secondary metabolites and hydroxamic acids (BXZs) such as benzoxazolin-2-one (BOA), 2-hydroxy-7-methoxy-1,4-benzoxazin-3-one (HMBOA), 2-hydroxy-1,4-benzoxazin3-one (HBOA) and 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) in wheat are considered to be phytotoxic and exhibit a role in plant defense against weeds. Here, we evaluated the effect of proximity of two weeds, *i.e.*, common purslane (*Portulaca oleracea*) and annual ryegrass (*Lolium rigidum*), on BOA/HMBOA/HBOA/DIBOA production in wheat cv. 'Ursita' seedlings under water agar culture in glass beakers to identify whether the breeding of modern wheat varieties with higher concentrations of these compounds could ensure plant-mediated weed control.

Surface-sterilized pre-germinated seeds of 'Ursita' were placed in 3 rows in one half of the agar surface in a glass beaker (2 L) containing 240 mL of 0.3% nutrient-free water agar media (previously autoclaved) and kept in a growth cabinet maintaining 25/15°C day and night temperature, respectively; and 13/11 h light/dark period. Following a week of growth, surface-sterilized seeds of *L. rigidum* or *P. oleracea* were placed in the same water agar medium on the other half area of the glass beakers and separated by a piece of pre-autoclaved white paperboard. The treatments (wheat + weeds) and respective control beakers (wheat alone) were prepared. After one week of co-cultivation, the substrate was removed and the roots were washed under tap water. After harvesting, 250 mg of shoots or roots of 'Ursita' were independently lyophilized, with pestle and mortar to make fine powder. Liquid nitrogen was added for pulverization and lyophilization, and 1 mM HCl (9 mL) was added to macerate the sample. Afterwards, the whole solution was transferred into a vial, sonicated for 15 min (Branson SINIFIER 250; microTip limit, output 3) and centrifuged at 20,000 rpm at 10°C for 15 min (SORVALL RC 5B Plus, Du Pont). The supernatant was collected and extracted three times with organic solvent, *i.e.* diethyl ether (DE). Quantification of hydroxamic acids and polyphenols were carried out using liquid chromatography mass spectroscopy (LC-MS).

We found that DIBOA was not detected in wheat when grown alone but when grown in association with the monocot and dicot weed. BOA, HMBOA, HBOA and DIBOA was significantly increased when wheat was co-cultured with either weed species and roots of both weeds and wheat were not in contact with each other (Table 1). When wheat was co-cultured with *L. rigidum* (planting density 10:10), higher quantities of BOA (75.3 mg·kg$^{-1}$), HBOA (186.9 mg·kg$^{-1}$) and HMBOA (1991.5 mg·kg$^{-1}$) were quantified from the shoot tissues. Similarly, BOA, MBOA and HMBOA demonstrate higher concentrations of 112.2 mg·kg$^{-1}$, 732 mg·kg$^{-1}$, and 5319 mg·kg$^{-1}$, respectively, in root tissues when grown in association with *L. rigidum* at the same plant density. We concluded that weeds were a source of biotic stress to wheat when grown in proximity to it, and the stress resulted in production of higher levels of BXZs in wheat seedlings, although the concentration varied with wheat seedling density. There are certain hydroxamic acids that only appear when weed is present, indicating that there are metabolic processes that take place in the wheat cells only when it feels attacked by a weed. Therefore, the synthesis and exudation of DIMBOA/HBOA in wheat seedlings appears to be an active metabolic process influenced by the environment, particularly the presence of weeds. Allelochemicals can be considered an important part of ecological weed management, but it is necessary to understand the class of allelochemicals and their possible mechanisms of action. In the present study, we quantified the hydroxamic acids and their subsequent distribution in plant tissues (roots and shoots) as well as from the root rhizosphere, in the presence and absence of weeds.

## Keywords

Allelopathy · enhanced exudation · wheat seedling · root · *Triticum aestivum* · weed

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**Table 1** Quantification of hydroxamic acids of diethyl ether (DE) extract from different plant tissues (shoots, roots) of wheat cv. 'Ursita' and growth medium (agar). Quantities are expressed in µg·kg$^{-1}$ dry weight as means ± standard deviation ($n$ = 3). Means followed by different letters within a column show significant difference according to Tukey's HSD test ($p \leq 0.05$).

| Treatment | Sample | DIMBOA | BOA | MBOA | HBOA | HMBOA | DIBOA |
|---|---|---|---|---|---|---|---|
| Ursita | Shoots | 14615 ± 2429 a | 14.9 ± 1.3 a | 1768.8 ± 140.1 a | 30.3 ± 4.1 a | 1171.8 ± 172.8 a | - |
| | Roots | 5622 ± 324 c | 6.4 ± 0.2 b | 374.2 ± 8.2 b | 15.9 ± 0.9 c | 476.4 ± 45.1 c | - |
| | Agar | 2654 ± 539 b | 7.4 ± 2.8 b | 202.5 ± 46.3 c | 26.4 ± 9.0 b | 563.0 ± 91.6 b | - |
| Ursita + *Lolium* (10:10) | Shoots | 12242 ± 4233 c | 75.3 ± 24.5 b | 917.6 ± 223.2 a | 186.9 ± 70.5 b | 1991.5 ± 611.3 b | 1.23 ± 0.4 a |
| | Roots | 4172 ± 758 c | 112.2 ± 40.1 a | 732.0 ± 178.2 b | 348.2 ± 150.3 a | 5319.1 ± 1821.3 a | 1.17 ± 0.4 a |
| | Agar | 7954 ± 657 b | 11.8 ± 0.8 c | 423.1 ± 6.3 c | 48.1 ± 4.0 c | 1090.7 ± 83.5 c | 1.42 ± 0.1 a |
| Ursita + *Lolium* (15:10) | Shoots | 5793 ± 123 a | 66.8 ± 17.7 a | 584.3 ± 193.8 a | 181.3 ± 89.0 a | 2572.7 ± 239.6 a | 0.98 ± 0.3 a |
| | Roots | 76 ± 32 b | - | 6.6 ± 2.9 b | - | 29.0 ± 5.7 b | - |
| | Agar | 33 ± 25 c | - | 3.0 ± 0.2 c | - | 29.6 ± 7.3 b | - |
| Ursita + *Portulaca* (10:10) | Shoots | 12590 ± 499 b | 21.9 ± 0.8 b | 319.0 ± 3.6 c | 209.6 ± 12.1 b | 3131.2 ± 130.8 b | 0.78 ± 0.0 a |
| | Roots | 19735 ± 257 a | 63.6 ± 0.8 a | 373.0 ± 5.7 b | 304.5 ± 6.3 a | 3371.5 ± 26.1 a | 1.2 ± 0.0 a |
| | Agar | 4402 ± 1835 c | 14.7 ± 4.7 c | 430.9 ± 19.8 a | 34.3 ± 14.3 c | 947.8 ± 70.9 c | 0.79 ± 0.3 a |
| Ursita + *Portulaca* (15:10) | Shoots | 12823 ± 568 a | 22.8 ± 1.0 a | 615.0 ± 30.7 a | 104.1 ± 5.0 a | 1613.8 ± 49.0 a | 2.64 ± 0.1 a |
| | Roots | 36 ± 11 c | - | - | - | 23.2 ± 7.0 c | - |
| | Agar | 46 ± 11 b | - | - | - | 29.1 ± 12.5 b | - |

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# Diversity of spelt and common wheat grown under different management based on their bioactive component composition

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## Abstract

Wheat and other cereals are important sources of dietary fibre and antioxidants. Most of the bioactive components are found in the outer layers and aleurone layers of the seed, but also the flour contains some. The major dietary fibre components in the wheat grain are cell wall polysaccharides such as arabinoxylan and β-glucan, which account for about 70% and 20%, respectively, of the total cell wall polysaccharides in the starchy endosperm. Barley and oats are rich in β-glucan, while wheat and rye are richer in arabinoxylan. Arabinoxylan has two forms, *i.e*. water-soluble (WE) and insoluble (WU) fractions, which differ in their health benefits. Antioxidants (*e.g*., alkylresorcinol) delay or inhibit oxidation processes. Alkylresorcinol is formed in the organism and acts against free radicals, which are responsible for the cell degradation.

Spelt (*Triticum spelta*) is often supposed to be healthier than common wheat (*T. aestivum*), but this assumption has never been confirmed based on the compositional properties of the grain. However, the environment and field management practices could also influence grain composition. The current study evaluated differences in the composition of bioactive components between spelt and wheat, as well as the effects of growing location and field experimental practices. Five wheat and five spelt varieties were grown for three years (2019-2021) at two sites in Hungary (M, conventional; O, organic). The organic site was an experimental field, where no artificial fertilisation and chemical treatment was applied for at least three years. The total amount of mixed-linkage β-glucan was determined according to the ICC166 Standard Method using the Megazyme assay kit (Megazyme, Bray, Ireland). Total and water-extractable pentosans, of which arabinoxylan (AX) is the major component, were determined by the colorimetric method. Alkylresorcinol content was measured by spectrophotometry.

The results showed that the main fibre of wheat, arabinoxylan (AX) content, was higher in wheat than in spelt, but there were no differences between conventional and organic growing sites. The water-extractable fraction of arabinoxylan (WEAX) was also higher in common wheat than in spelt, with no differences due to field management practice (conventional vs. organic). β-Glucan, present in lower amounts than arabinoxylan, showed also higher amounts in common wheat than in spelt. The highest mean value was found for both species at the organic site. However, variation was high in the wheat samples grown on the organic site. The content of alkylresorcinol, which has antioxidant activity, was similar in spelt and wheat, with higher variation in wheat (Fig. 1). Consequently, the content of dietary fibre (arabinoxylan, β-glucan) was lower in spelt than in common wheat under both conventional and organic growing conditions. No difference was found in the mean alkylresorcinol content of spelt and common wheat at any of the sites. Organic field management resulted in significantly higher mean β-glucan content for both species compared to the conventional site.

## Keywords

Alkylresorcinol · arabinoxylan · beta-glucan · diversity · organic farming · *Triticum aestivum* · *Triticum spelta*

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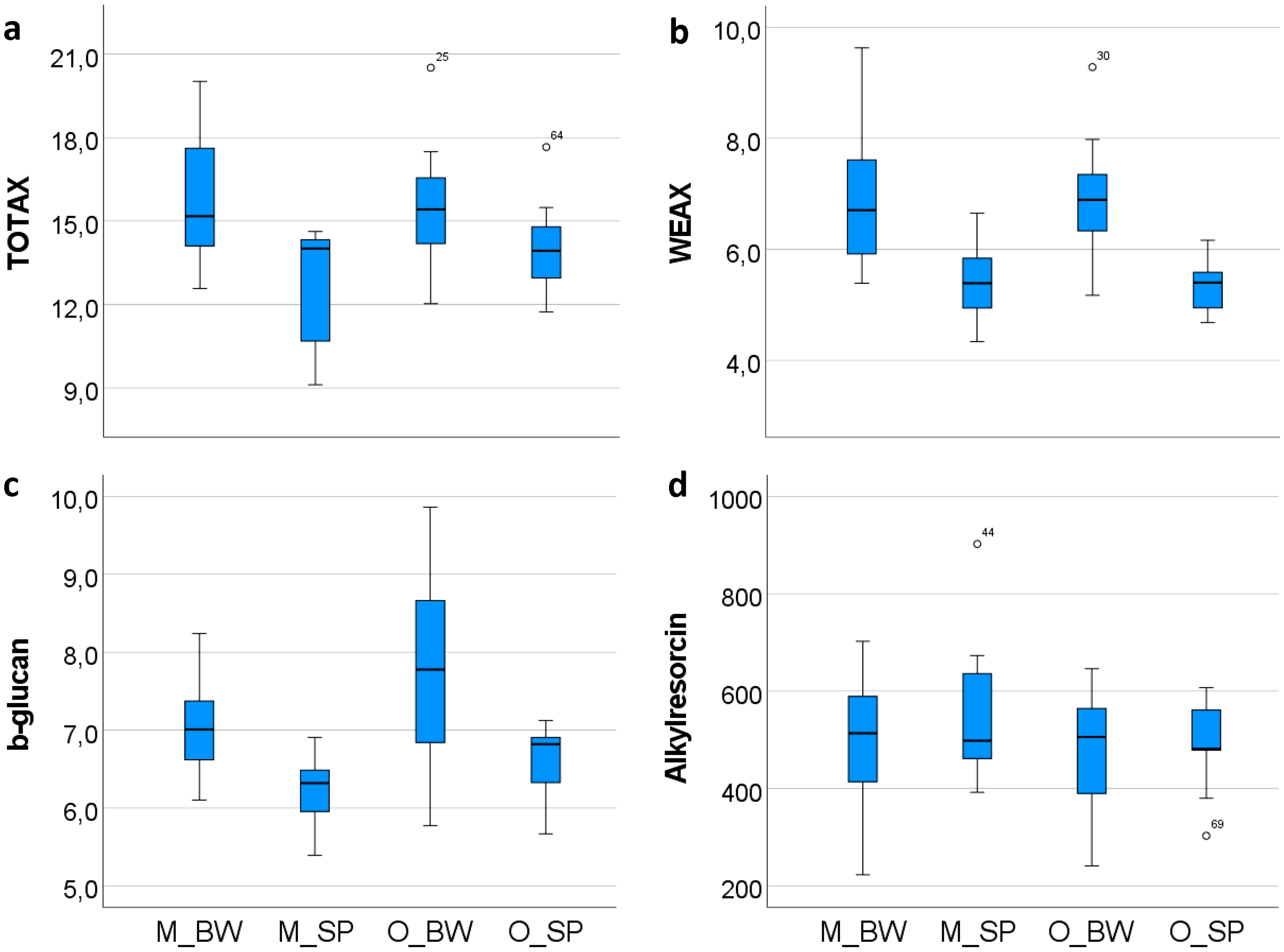

**Figure 1** Variation in bioactive components of common wheat (BW) and spelt (SP) grown under conventional (M) or organic (O) management in Martonvásár, Hungary, from 2019 to 2021. **a** Total-arabinoxylan content (TOTAX; mg·g$^{-1}$); **b** Water extractable-arabinoxylan content (WEAX; mg·g$^{-1}$); **c** β-glucan content (mg·g$^{-1}$); **d** alkylresorcinol content (µg·g$^{-1}$)

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# Identification of useful traits for organic soybean breeding in limiting and changing agro-climatic conditions

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## Abstract

Organic farming is practiced on over 72 million hectares of agricultural land worldwide. Soybean (*Glycine max*) is cultivated organically on 644 323 ha. Each year, the selection of the right soybean varieties for their fields is one of the most challenging management decisions that farmes must undertake. Soybean breeding companies continuously offer varieties from their breeding programs that can meet the various needs of farmers, industry and consumers. The growing demand for soybean varieties suitable for organic farming is fostering breeding activities to better fulfil the specific requirements of the organic value chain participants.

Within the ECOBREED project, characterisation of a soybean working collection is carried out across contrasting environments in three countries, *i.e*. Austria, Romania and Serbia, which will enable the identification of useful traits (variation) and the level of local adaptation of genotypes. The following traits are assessed: yield and yield components, crop growth related traits, grain quality traits. The main priority of the project is the creation and selection of new soybean breeding material suitable for organic farming and breeding. The ECOBREED trial network includes in total 208 accessions, subdivided in two maturity groups, and is tested in the thee countries from 2019 to 2022. Here, first selected results are presented. Due to a severe weed infestation in the early growing period and severe drought later, the trials conducted in Romania in 2020 were excluded from statistical analysis.

One important trait for organic breeding is canopy cover, as a surrogate for canopy light interception, and on the other hand as potential weed competitiveness trait. The importance of canopy cover of soybean in weed competitiveness is not clear. Some stu-

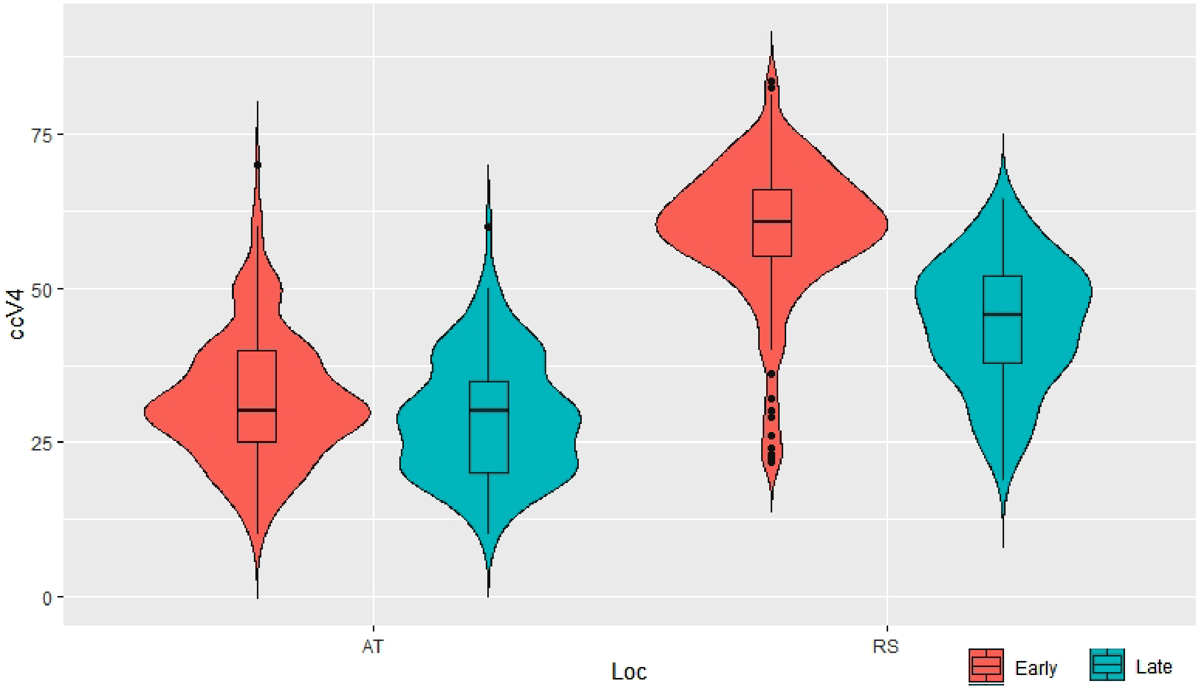

**Figure 1** Variability in canopy cover of 208 soybean genotypes tested in 2020 in Gleisdorf, Austria (AT) and Novi Sad, Serbia (RS). The nursery was subdivided into two trials at each location according to maturity groups.

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dies indicate that this trait is important for weed competitiveness, whereby other studies have not confirmed a relationship between these traits. All studies were done on a relatively small number of genotypes and the reported variability in this trait was low. In this study, canopy cover was screened during the V3-V4 phase of development using the Canopeo App.

In general, canopy cover was higher in Serbia than in Austria (Fig. 1), mainly due to higher temperatures during the vegetaton period. Considerable variation in canopy cover was observed at both locations and in both maturity groups. In Austria, the mean canopy cover of the early and late genotypes was similar, while in Serbia early genotypes showed a higher canopy cover compared to late genotypes. In each group, it was possible to identify genotypes with a high canopy cover (20% higher than the group mean) that represent soybean genotypes with a potential good weed competitiveness. Future studies should provide more information about the importance of this trait in the relationship between soybean and weeds.

**Keywords**

Canopy cover · *Glycine max* · organic breeding · weed competitiveness

**Acknowledgements**

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# ECOBREED participatory trials for organic soybean production in Serbia

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## Abstract

Due to the rising demand for organic food products and increased availability of organic products, global production of organically grown crops has increased. In 2020, organic agriculture was practised in 187 countries worldwide, on 72.3 million ha of agricultural land by at least 3.1 million farmers. The area of organically managed soybean worldwide was 644 323 ha. Soybean production is of great importance since the soybean grain is used in various industries, including human as well as animal nutrition and pharmaceutical production. Soybean has a special place in organic cultivation because it brings different agronomic benefits to farmers and it is considered a niche product for feed and food use. Furthermore, soybean is primarily a climate-smart crop that conserves soil fertility for increased productivity of other important field crops, and its place in organic production is very valuable. The importance of soybean comes from the exceptionally favorable grain chemical composition (≈40% protein, ≈20% lipids). Soybeans are relatively easy to produce under organic conditions due to available production technology. One of the most important tasks for farmer is to select soybean varieties for specific production conditions.

On-farm variety trials were set up in Serbia as part of the ECOBREED project. Aim of these trials was to support farmers in the selection of new varieties for their respective pedo-climatic zones and locations. Organic farmers typically have extensive knowledge of crop traits required for optimal agronomic performance, as well as quality requirements required by the industry and consumers. The trials served as a baseline for the following season. During the growing period organic farmers were actively involved in evaluations, and they were trained to be able to select soybean varieties that are better suited to their specific area and growth conditions.

The trials were set up in 2021 on five locations: (i) Rimski Šančevi (45°30'59.5"N, 19°59'26.3"E); (ii) Šuljam (45°05'20.2"N, 19° 40'14.5"E); (iii) Bela Crkva (44°57'27.1"N, 21°19'11.2"E); (iv) Čurug (50°36'71.3"N, 42°06'20"E); and (v) Banatsko Karđorđevo (45° 35'26.5"N, 20°33'42.2"E). Soybean varieties from the 00, 0, I, and II maturity groups were evaluated in the trial network. The varieties were 'Xonia' from Saatzucht Gleisdorf, Austria, and 'NS Altis', 'NS Apolo', 'NS Mercury', 'Rubin' and 'Zora' from the Institute of Field and Vegetable Crops, Novi Sad, Serbia. The crops were sown in early April and harvested in September. The experiment was set up as strip trial with a plot size of 300 m$^2$ per variety. Inter-row management was performed twice during the growing season in May as well as manual weed control. The trials were harvested in September according to the maturity of the varieties. From each plot, three samples from central rows were taken for yield, protein and oil content measurements.

The varieties from the 00 to II maturity groups showed different adaptability to the specific conditions of the involved farms. Grain yield was between 1.96 and 4.61 t·ha$^{-1}$ (Fig. 1), while protein content was within the range of 33% to 44% d.m. The lowest yields (1.96 - 2.88 t·ha$^{-1}$) were recorded at Bela Crkva which is not a preferable soybean growing region. Highest yields for all varieties (3.48 - 4.53 t·ha$^{-1}$) were observed in Čurug.

## Keywords

*Glycine max* · organic farming · protein · yield

## Acknowledgements

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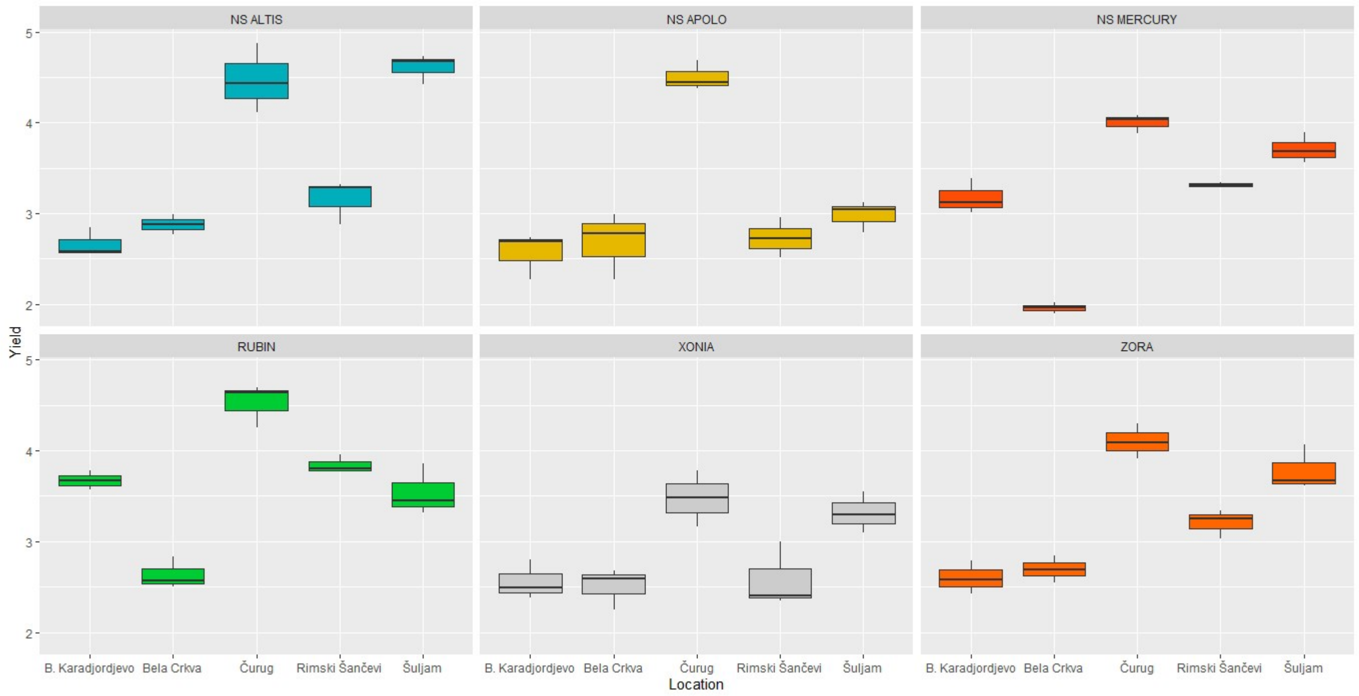

**Figure 1** Soybean yield (t·ha$^{-1}$) of six varieties (*i.e.*, 'NS Altis', 'NS Apolo', 'NS Mercury', 'Rubin', 'Xonia', 'Zora') in the ECOBREED farmers participatory trials at five locations in Serbia 2021.

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# *Bruchus rufimanus* - a pest complicates domestic legume seed production

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## Abstract

Faba bean (*Vicia faba* L.) is an appreciated domestic legume whose cropping area increased significantly in Germany and other European countries in the last years. However, due to difficulties in seed production the demand for high quality seeds cannot be supplied every year. The reasons for it is the generally high vulnerability of the seeds to mechanical damage and the increased infestation with the broad been beetle (*Bruchus rufimanus* Boh.). Contrary to the general assumption seed quality is not affected directly by the beetle, but indirectly because of higher imbibition speed and pathogen susceptibility through the holes in the seeds which the beetles leave after emergence. Since there are currently no effective control methods against these bruchids in the fields, new strategies must be developed and tested for organic and conventional cropping systems.

A series of field trials was conducted from 2019 to 2021 with three organic and conventional sites each. The test sites were located in Futterkamp and Hohenlieth in Schleswig-Holstein (North Germany), in Puch in Upper Bavaria (South Germany), and in different sites in Saxony and Lower Franconia (Central Germany). Each field trial was completely randomised and replicated four times. In the organic field trials plant protection products based on neem oil (NeemAzal®-T/S) were applied and adult individuals of *Lariophagus distinguendus* were introduced as natural enemies in open land and housed plots. In the conventional field trials the effectivity of insecticides (Karate Zeon®) and late sowing dates were investigated. Each trial variant was sown with bruchid-infested and bruchid-free seeds. To study the effects of emigrating and immigrating seed beetles, plots of each trial variant in each field trial were additionally covered with an insect exclusion net-tunnel. Besides plot yield and thousand grain weight, germination capacity following ISTA (International Seed Testing Association) rules and the percentage of infested seeds of each plot was determined. In 2021 an additional field trial following a push-pull-approach with semiochemical repellent and attractant substances was conducted to develop new control strategies against *B. rufimanus*.

Neither in the organic nor in the conventional field trials satisfying pest control was achieved in the open, uncovered plots. The significant reduction in bruchids due to the parasitization of *Lariophagus distinguendus* in the netted plots compared to open plots shows the high dilution effect of natural enemies under normal field conditions. The high population size of natural enemies needed to compensate for this dilution effect inhibits the applicability in fields due to technical as well as economic reasons. Independent of the infestation of the original seeds, the highest mean germination capacity across the three growing seasons was obtained for plots treated with insecticides, either the organic (NeemAzal®-T/S) or conventional (Karate Zeon®) type. A significant ($\alpha$=0.05) effect of other control strategies was present only in a few trials and the minimum requirements for seed quality were not reached in the trials in Central and South Germany. The heterogeneity between trial sites is due to environmental conditions such as precipitation, temperature rise in spring and the occurrence of natural enemies in the local fauna. Legal minimum germination capacity of faba bean seeds (i.e. 80% in Germany) was reached even in untreated plots in the North German field trials. Late sowing and the consistent delay of faba bean flowering reduced the degree of bruchid infestation. Contrary to the trend in the overall data, mean germination capacity was not significant higher than in untreated plots despite lower degrees of infestation. A probable reason is the shortened vegetation period combined with water shortage, as happened in 2020. The comparisons between netted and open plots and plots sown with beetle free or infested seeds shows that the predominant part of damaged seeds is caused by bruchids immigrating to the fields and the damage of beetles leaving from infested seeds in the fields can be neglected: the mean difference between open and housed plots was 11%, while the difference between beetle free and infested seeds was <1%. With regard to germination capacity a significant difference between beetle free and infested seed was observed only in some individual field trials, but not consistently for the pooled data.

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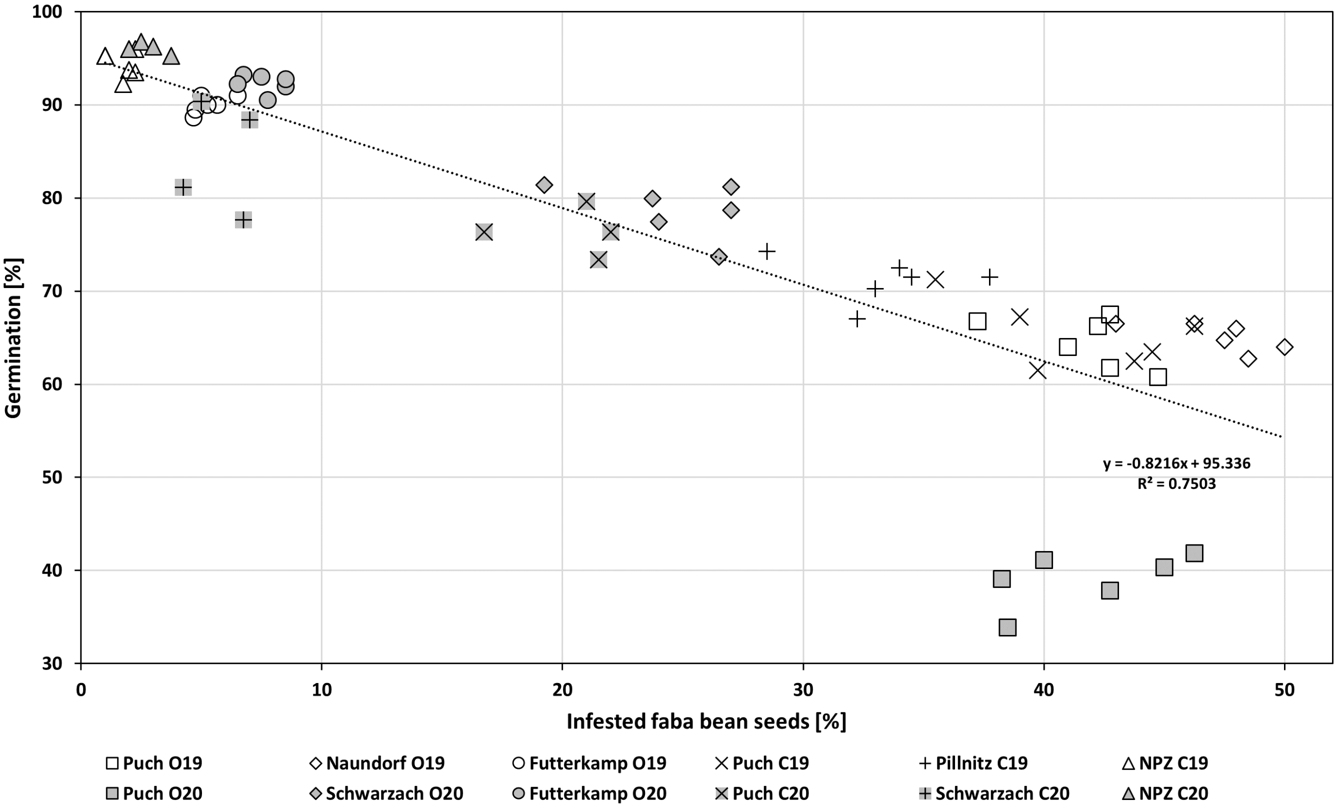

**Figure 1** Correlation between germination capacity and seed infestation. Two-year means (2019-2020) of open plots; different sites are displayed by different symbols, 2020 trials by filled symbols; management is abbreviated as C and O for conventional and organic, respectively.

**Keywords**

Broad bean beetle · faba beans · germination capacity · seed quality · *Vicia faba*

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# Breeding climbing beans for intercropping with maize

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## Abstract

The cultivation of maize and beans as intercrops has been practiced in Mesoamerica for centuries. Nowadays breeding for these two crops is generally conducted in pure crop stands. Aim of this intercropping system is to use the positive effects of both components, the performance of maize and the nitrogen fixation of beans. In Europe a maize-bean intercropping system for silage production is being established. For maize, a very lodging tolerant variety is needed. Suitable bean varieties are cold-tolerant, late-ripening, have small seeds and a low level of phytohemmagglutinin. Major selection traits in this intercropping system are high total biomass yields, high protein contents and a strong bean stand. Yield trials were conducted in Germany in multiple locations with a maize-bean ratio of 2:1. Over the last three years of selection, biomass yield and protein content of the intercropping treatments could be increased substantially. Especially under low nitrogen conditions, maize and bean intercropping is highly advantageous to pure maize cropping and shows the potential of a more sustainable maize production.

## Keywords

Intercropping · mixed cultivation · pole bean · *Phaseolus vulgaris* · *Zea mays*

## Introduction

Milpa, the cropping system where different crops (maize and bean among others) are combined, is known from Central America since centuries (Diemont & Martin, 2009; Nigh & Diemont, 2013). Since more than 70 years intensive maize breeding is done for pure crop cultivation (Lee & Tollenaar, 2007). Since the domestication of common bean, several traits have been adapted for vegetable usage (Koinange *et al.,* 1996). Nowadays, huge areas are cultivated with a small number of crops. In Germany, the acreage of maize for silage in 2020 was 2.3 million hectares (Destatis, 2021). The breeding program of pole beans for intercropping with maize is going on since 2013 at Sativa Rheinau. The evaluation of the beans takes place on poles and in combination with maize in parallel. Principles of the ecosystem intercropping are reciprocal positive interference of the mixing components (Baudoin *et al.*, 1997), resource use efficiency (Hauggaard-Nielsen *et al.*, 2009; Latati *et al.*, 2016) and reduced area of monoculture. Aim of the trials is the selection of bean lines with the best mixing ability with maize and the greatest performance concerning dry matter yield and protein content of the mixture. Necessary requirements for other agronomical traits are for example small seed size and low level of phytohemmagglutinin content.

## Material and methods

### *Plant material*

In the nursery, the beans grow on poles with a distance of at least 50 cm to the next pole. The beans are separated reguarly to evaluated single plants. For the intercropping with maize, a variety which is highly lodging tolerant is required. In 2018, 2019 and 2020 cv. 'KWS Figaro' was used for the trials. Bean lines of young generations which have been selected on the pole in the nursery are tested in combination with maize. The best bean varieties in the one year are selected for replicatied trials in the following year. Bean varieties which are already available on the market or varieties which are proven for their high performance over years are used as check varieties.

### *Methodology*

The general criteria of the beans can be evaluated in the nursery. Cold tolerance of beans is tested by choosing an early sowing date (end of April) under field conditions. The optimum maturity results from the point of silaging. A high amount of pods and leaves at harvest time is requested. A rough estimation is done in the nursery. The additional information or confirmation is supplied by the intercropping trial. After the harvest of dry beans, the thousand seed weight (TSW) is evaluated.

To evaluate the general mixing ability (GMA) an exact trial is organised where the beans are tested in combination with maize. Depending on the year, the trial was located in four to six locations in Germany. Since 2020, a location with low nitrogen conditions is included in the trial network. In 2020, a total N content of 60 kg N·ha$^{-1}$ was targeted. The plot contains two rows with each 5 or 6.2 m length depending on the location. Each genotype is tested in two replications per location. On the testing plots, 8 plants of maize and 4-5 plants of beans per m² are sown. The reference plots should simulate a cultivation of pure maize. Therefore, 10 plants

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of maize per m² are sown. During the growing period the genotypes are evaluated visually. The harvest is done by a conventional maize chopper where each plot can be harvested separately. Protein data are gained from a representative sample in the laboratory. Fresh matter yield, dry matter content and dry matter yield are evaluated. Moisture content is assessed by NIRS. Protein content is determined by the Kjeldahl method using a N conversion factor of 6.25. Because the pure maize cultivation symbolises a benchmark, the results are related to pure maize plots which are respectively set to 100.

## Results and discussion

In the last years, a thousand seed weight of bean breeding lines between 200 and 300 g was achieved. Due to early and deep sowing, vigorous and cold-tolerant plants could be selected. The genetic material was shifted to more late-ripening genotypes by doing recurrent visual selection in September. Early-ripening varieties cannot compete with late-ripening with respect to dry matter yield (Fischer *et al.,* 2020).

The results of the intercropping trials confirm the increase of the performance of the bean lines over the years (Fig. 1). In 2018, dry matter of the intercropping plots was on average less than 90% relative to the pure maize plots. In 2019, the bean-maize-plots exhibited an average value of 93% relative to pure maize, and in 2020, the intercropping plots averaged around 96% relative to the pure maize plots. Similarly, protein content and protein yield were increased over the years. In 2020, crude protein yields exceeding pure maize could be obtained.

Under low N conditions the role of the bean was much more pronounced. Maize-bean plots clearly outperformed pure maize stands in all three major traits, *i.e.*, yield, protein and protein yield. For dry matter yield, values up to 115% relative to maize could be obtained. For protein, this increase was even much higher and reached up to 160% relative to pure maize for crude protein yield (Fig. 2).

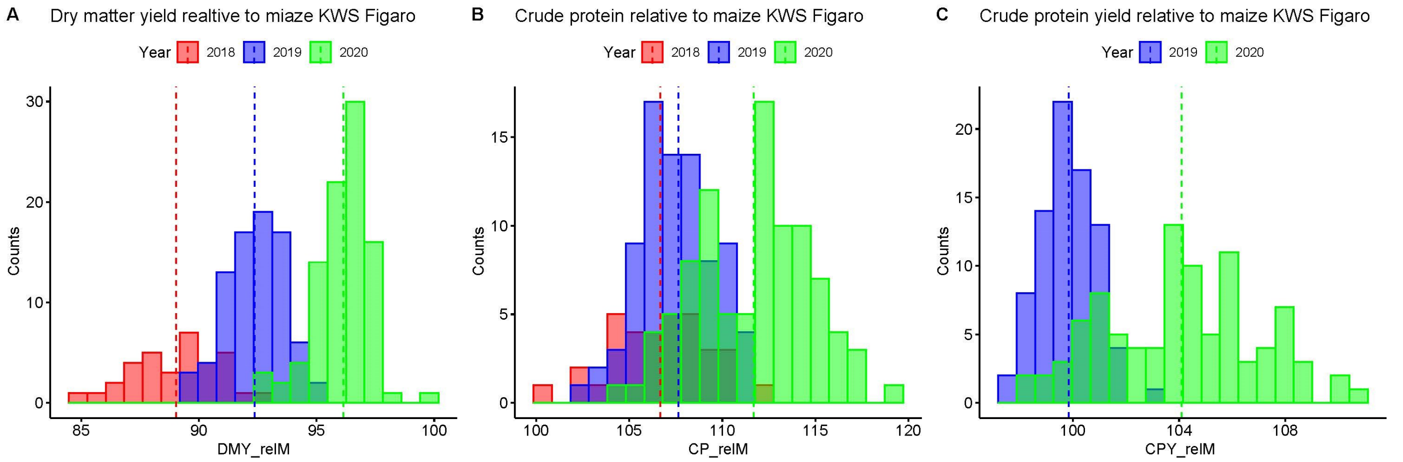

**Figure 1** Selection progress of 3 years under normal N conditions: **a** dry matter yield relative to maize; **b** crude protein content relative to maize; **c** crude protein yield relative to maize.

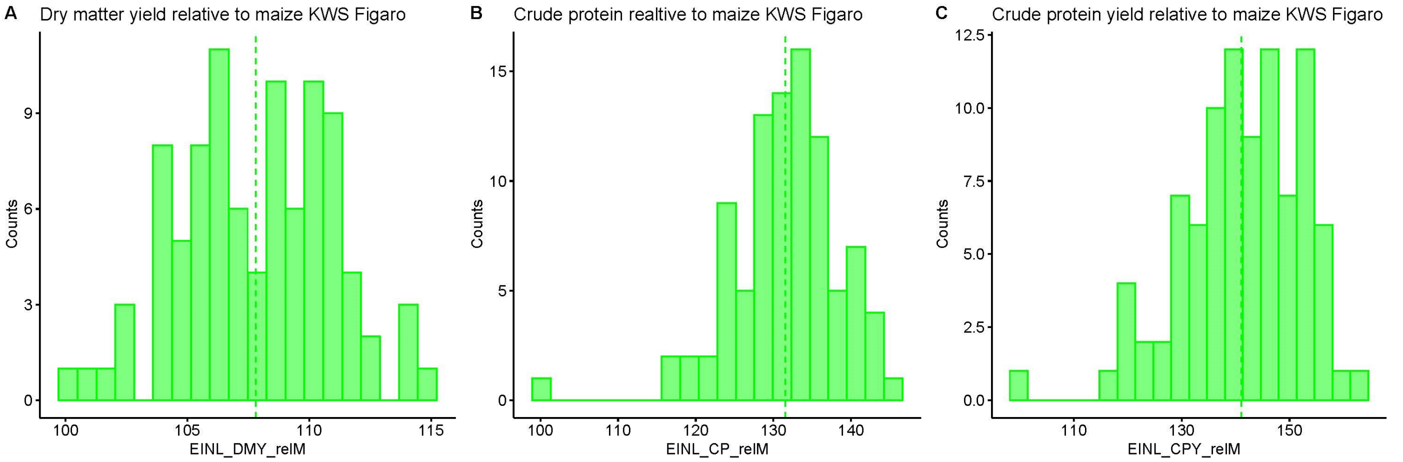

**Figure 2** Performance under low N conditions in year 2020: **a** dry matter yield relative to maize; **b** crude protein content relative to maize; **c** crude protein yield relative to maize.

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# Selection of advanced potato breeding lines at the Agricultural Institute of Slovenia within the ECOBREED project

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## Abstract

Potato breeding has a long tradition at the Agricultural Institute of Slovenia. The main focus of the program is to breed varieties adapted to Slovenian growing conditions. Due to high virus infection pressure, breeding for extreme resistance to potato virus Y (PVY) was the first choice of the new breeding program which started in 1993. Breeding for efficient durable late blight (*Phytophtora infestans*) resistance was the next step that was added to the program in 1998. Within the framework of this program, an annual volume of about 10,000 seedlings is achieved. Crossings with selected parents are done in greenhouse on plants grown on the brick. Mass selection against PVY is performed after artificial inoculation at the seedling stage, followed by selection for important qualitative and quantitative traits in subsequent 10 field generations. Selection of desirable traits includes several parallel methods that depend on the breeding objectives: (1) selection for genotypes resistant to PVY and late blight, (2) qualitatively inherited characteristics (*e.g.*, shape of tubers, depth of eyes, skin and flesh color, stolon length end plant habit), (3) quantitatively inherited characteristics (*e.g.*, tuber yield, number of tubers, tuber size, dry matter), (4) utilisation traits (*e.g.*, boiling, baking, frying).

Selection of advanced breeding lines suitable for organic agriculture is one of the goals of the ECOBREED project. It includes crossings of genotypes resistant to late blight and PVY, pyramiding of several resistance (*R*) genes within one genotype, introduction of earliness in resistant progenies, use of molecular markers for selection of *R* genes and selection of advanced lines on organic soils from the 3rd year of selection on the field. Extreme *R* genes against PVY from *Solanum stoloniferum* ($Ry_{sto}$) and *S. chacoense* ($Ry_{chc}$) are utilized in the program. Major late blight *R* genes were introduced from *S. demissum, S. bulbocastanum, S. chacoense, S. phureja* and from variety 'Sarpo Mira' (*i.e.*, *Rpi-Smira2/R8* gene). The long term goal is the registration of organic varieties through organic variety trials.

In 2020, 165 potato plants were grown on the brick in the greenhouse at KIS for new crosses between resistant parents and high quality varieties. 19 parents were late blight resistant and 17 parents were PVY extreme resistant, others were commercial high quality varieties. By the end of July, 31 successful crosses of LB resistant and susceptible parents and 16 crosses of both LB resistant parents were made.

In 2021, selection of clones on organic fields started at KIS. For the purpose of variety development, 229 clones from the crossing years 2017 to 2020 were picked for selection under organic conditions in 2021. 47 of advanced clones were late blight resistant, proven by molecular markers (Table 1). All of them showed also extreme resistance to PVY. For all generations, 4 tubers per clone were planted in 2021 together with check varieties 'KIS Slavnik', 'Alouette', 'Carolus' and 'KIS Kokra'.

For the purpose of *R* gene pyramiding, marker assisted selection (MAS) was applied in 2018 and 2019 on 2,100 genotypes from 12 progenies of crosses with resistance parents carried out in 2017. In 2020, remaining 327 late blight resistant genotypes were planted in a conventional field as 4 hill plots in Lahovče and 92 advanced clones were selected for planting in an organic field in 2021.

The selection of advanced breeding lines suitable for organic agriculture was successful. In both respects, variety development and pyramindig of *R* gene, new combinations of *R* genes were successfully made: (1) *Rpi-Smira2/R8* and $Ry_{chc}$ from cv. 'Sarpo Mira' × *Rpi-Chc1* from cv. 'Carolus'; (2) *Rpi-Smira2/R8* and $Ry_{Chc}$ from 'Sarpo Mira' × *Rpi-Vnt1.1* from cv. 'Alouette', (3) *Rpi-Smira2/R8* and $Ry_{Chc}$ from 'Sarpo Mira' × *Rpi-BLB2* from cv. 'Toluca' (4) *Rpi-Smira2/R8* and $Ry_{Chc}$ from 'Sarpo Mira' × $Ry_{Sto}$ from other varieties, (5) *Rpi-Chc1* from 'Carolus' × $Ry_{Chc}$ and $Ry_{Sto}$ from other varietiess, (6) *Rpi-Vnt1.1* from 'Alouette' × $Ry_{Chc}$ and $Ry_{Sto}$ from other varieties.

**Table 1** Molecular marker used in potato breeding programme at KIS (Agricultural Institute of Slovenia).

| Trait | Resistance genes | Molecular marker |
|---|---|---|
| *Phytophtora infestans* | *Rpi-Smira2/R8* | R8-UTR |
| | | R8-CDS |
| | *Rpi-vnt1.3* | Phu6 |
| | *Rpi-vnt1.1* | LK69/70 |
| | *Rpi-chc1* | MN586/587 |
| Potato virus Y | *Ry-sto* | SCAR Rysto4 |
| | *Ry-chc* | Ry186 |

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**Keywords**

Organic agriculture · resistance genes · potato virus Y · potato late blight · molecular markers

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# (Pre-)Breeding of potatoes suitable for organic farming in the ECOBREED project

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## Abstract

The cultivation of potato (*Solanum tuberosum* L.), the third most important food crop worldwide, is noteworthy affected by the potato pathogen *Phytophthora infestans* (Mont.) de Bary, the causal agent of potato late blight. In conventional production systems, yield losses caused by late blight can be be limited by using synthetic pesticides. However, such treatments are prohibited in organic agriculture. Therefore, the only way for a sustainable control of the disease in organic farming is growing late blight resistant cultivars. Although the number of potato cultivars, that are proposed as suitable for organic agriculture is increasing, there is a lack of reliable information about its level of field resistance against *P. infestans* and sources of resistance present in these cultivars. Such information is, however, crucial for both organic farmers and organic breeders. Thus, within the frame of the Horizon 2020 ECOBREED project, potato cultivars were assessed with respect to a broad spectrum of traits which makes them suitable for organic farming, including late blight resistance under field conditions. To gain knowledge on resistance sources which are present in this material, all cultivars were screened with DNA markers linked to specific *R* genes against *P. infestans*.

The plant material consisted of 65 potato cultivars, which were selected for the ECOBREED potato working collection. The level of its field resistance against *P. infestans* was assessed on 6 hill plots with 2 blocks (replications) during two vegetation periods (*i.e.*, 2020 and 2021). Starting from the second half of June all cultivars were assessed once a week. The degree of infection of each plot was expressed as percentage of late blight-symptomatic canopy. Based on at least 5 scorings, the relative Area Under Disease Progress Curve (rAUDPC) was calculated for each cultivar. The rAUDPC values range from 0 to 1, with low values corresponding to high resistance. To verify the presence of particular *R* genes against *P. infestans*, all cultivars were screened with DNA markers linked to following six *R* genes: *R1, R2, R3a, R8, Rpi-chc1* and *Rpi-phu1*.

The resistance level in the ECOBREED working collection range from very high to very low, *i.e.*, mean rAUDPC values from 0 to 0.999. Based on these results, the cultivars were divided into ten resistant classes with steps of 0.1 rAUDPC (Fig. 1). Usually, only potato clones/cultivars with rAUDPC ≤0.2 (class 1 and 2 in our experiment) are considered as sufficiently resistant to survive and produce reasonable tuber yield without pesticide protection against *P. infestans*. In our experiment, the two most resistant classes (mean rAUDPC values 0 - 0.1 and 0.101 – 0.2) included 17 and 6 cultivars, respectively. The obtained results suggest that (considering exclusively its late blight resistance level) only 23 out of 65 (35.4%) potato cultivars could provide a reasonable level of protection against potato late blight, when grown by organic farmers. Six cultivars with mean rAUDPC values between 0.201 and 0.5 could by described as medium resistant to late blight. A successful growing of such cultivars under organic conditions strongly depends on environmental effects, in particular on weather conditions. The remaining 36 potato cultivars (>50% of the tested cultivars) are susceptible or even very susceptible to late blight. When such cultivars are grown under organic conditions without fungicides, they can be completely destroyed within 2 weeks, even if there is only a moderate infection pressure of *P. infestans*.

Screening of the potato cultivars with DNA markers, linked to *R* genes, showed that all examined *R* genes were present in the tested potato gene-pool. Most of these genes (*i.e.*, *R1, R2, R3a and R8*) were introduced from the wild species *S. demissum*. Genes *Rpi-phu1* and *Rpi-chc1* were introduced from *S. phureja* and *S. chacoense*, respectively. Two genes (*i.e.*, *R1* and *R3a*) were introduced into the cultivated potato gene-pool many years ago. Nowadays, they provide resistance against a rather narrow spectrum of *P. infestans* races. One examined gene (*i.e.*, *R2* and its homologs/orthologs) was also introduced into cultivated potato many years ago, but still provides resistance against an intermediate spectrum of races of the pathogen. Three genes (*i.e.*, *R8, Rpi-*

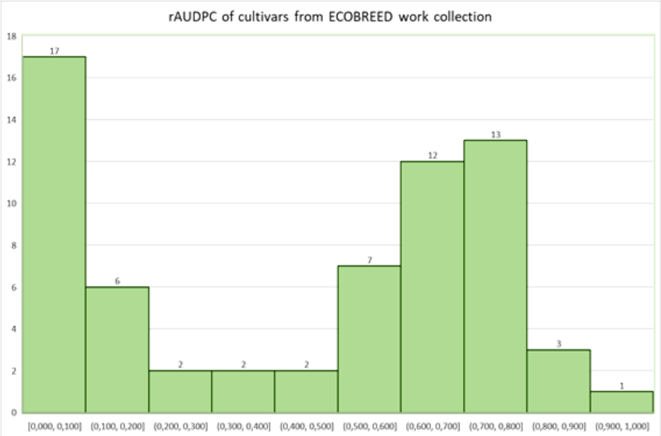

**Figure 1** Frequency of cultivars of the ECOBREED potato working collection in the 10 resistance classes against late blight (0 = highly resistant, 1 = highly susceptible).

Plich J, Tatarowska B (2022) (Pre-)Breeding of potatoes suitable for orangic conditions in ECOBREED Project. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 72. Jahrestagung 2021, 22-24 November, Raumberg-Gumpenstein, pp 93-94. University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-96-1

*chc1* and *Rpi-phu1*) were introduced into cultivated potato gene-pool quite recently and still provide highly effective protection against a broad spectrum of *P. infestans* races.

Results of our study showed that *R1* and *R3a* are present in cultivars of almost all resistance classes, except the most susceptible group. *R2* was present in four cultivars of class 1 (rAUDPC 0.0 – 0.1), one cultivar of class 2 (rAUDPC 0.101 – 0.2) and in one cultivar of group 4 (rAUDPC 0.301 – 0.4). Genes *R8, Rpi-phu1, Rpi-chc1* were present exclusively in cultivars of the most resistant class (rAUDPC 0 – 0.1). *R8* was present in 8 cultivars, while both *Rpi-phu1* and *Rpi-chc1* were present in three cultivars each. Such results confirm that genes *R1* and *R3a* are frequent in the potato gene-pool, but nowadays they are not effective anymore against *P. infestans* infection. However, some researchers suggest that such genes could enhance the effectiveness of other *R* genes. Although *R2* was introduced into the cultivated potato gene-pool long time ago, it is still quite rare in potato cultivars, and its effectiveness is surprisingly high. According to many authors, *R2* and its homologs/orthologs are still valuable sources of resistance in breeding programs. Gene *R8* was introduced into potato cultivars in the first decade of the 21st century for the first time. This gene provides high resistance against a broad spectrum of *P. infestans* isolates, but some authors note that its effectiveness could be background-dependent. Genes *Rpi-phu1, Rpi-chc1* were introduced into cultivated potato gene-pool very recently, and there is very little information about cultivars possessing these genes. Results of our studies clearly show that the genes *R8, Rpi-phu1* and *Rpi-chc1* provide a highly effective protection against current populations of *P. infestans.* Thus, they are very valuable sources of resistance for using in breeding programs.

There is still a scarcity of potato cultivars combining a high levels of resistance against *P. infestans* and a good level of other agronomic traits which are necessary for organic table potatoes. Identifying cultivars which possess various broad-spectrum resistance genes against potato late blight allows to use them as resistance donors in breeding programs. Currently, pyramiding of various broad-spectrum *R* genes in one cultivar is considered the best solution to breed potato cultivars with a high level of durable resistance against late blight. To realize such a breeding program, the access to a high diversity of resistance genes is a key element. After donors of such genes were identified, the next step in the ECOBREED project is to combine all three broad-spectrum resistance genes in new potato progenies. The selection of such clones with pyramided *R* genes will be performed by using DNA markers.

## Keywords

Late blight · marker-assisted selection · *Phytophtora infestans* · resistance breeding · *Solanum tuberosum* · wild relatives

## Acknowledgements

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# Pathological and yield components analysis of potato varieties potentially applicable for organic production

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## Abstract

The ECOBREED potato working collection was tested in 2021 in organic field trials in Hungary for their reaction to the main diseases Potato Virus Y, early blight and late blight. Additionally tuber yield and tuber defects were recorded. The results show a great genotypic variability in the investigated traits and, therefore, indicate a great potential for the selection and development of cultivars adapted to the conditions of organic potato growing.

## Keywords

*Alternaria solani* · resistance breeding · *Phytophtora infestans* · Potato Virus Y · *Solanum tuberosum* · tuber malformation

## Introduction

Potatoes are one of the most important staple foods (FAO 2008), but their successful cultivation, especially under organic conditions, is hampered by the frequent susceptibility of commercial varieties to biotic and abiotic stresses. To meet the increasing demands, cultivars with a wide range of adaptability to diverse environmental factors (Hassanpanah, 2010) and resistance against the most important pathogens and pests are needed (Forbes, 1999). As an alternative to intensive agricultural technologies that have the potential to endanger both the environment and consumers' health, there is a increasing demand for organic foods. The selection and breeding of varieties specifically designed for this purpose play a key role in ensuring the economics of organic crop production (Polgar *et al.*, 2016; Keijzer *et al.*, 2021).

In our experiments, we aimed to compare the varieties recommended by European potato breeders for organic cultivation in terms of productivity, resistance to pathogens, pests and abiotic stresses. We also examined how the individual results can be interpreted: based on what characteristics should we classify the varieties as most advised ones for organic production?

## Material and methods

Organic field experiments were set up in Hungary in 2021 under conditions that are highly predisposing to the physiological and pathological degeneration of potatoes, with a seed tuber that comes from being cultivated twice under organic conditions in Hungary (2019, 2020). The number of tested cultivars was 67, the size of the plots was 7 $m^2$ (row spacing 75 cm, plant spacing 30 cm, 30 plants per replicate, 4 replicaes). Weeding of experimental plots was done by hoeing 4 times in the season. Spinosad was used twice to control the potato beetle. The experiment was planted in mid-April and harvested in September. The incidence of the pathogens Potato Virus Y (PVY), *Alternaria solani* (early blight) and *Phytophtora infestans* (late blight) was recorded 4 times during the growing season by evaluating the symptoms on the foliage and assessing the severity and frequency of symptoms (1 very susceptible with severe symptoms, 9 no symptoms). At harvest, we recorded tuber yield ($t \cdot ha^{-1}$), size distribution of tubers, and the proportion of the marketable yield through the proportion of tubers having a size >4cm, and the number of tubers showing physiological disorders (malformation and cracking) or severe infections by *Streptomyces* sp. (common scab).

**Table 1** Reaction of the ECOBREED potato working collection to pathogens and abiotic stresses.

| | PVY | | | Late blight | | | Early blight | | | Tuber defects | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HR[1] | MR | S | HR | MR | S | HR | MR | S | HR | MR | S |
| Score | 8-9 | 5-7 | < 5 | 7 | 5-6 | < 5 | 8-9 | 5-7 | <5 | 0-20 | 21-40 | < 41 |
| Genotypes (*n*) | 30 | 15 | 22 | 3 | 24 | 40 | 41 | 19 | 7 | 22 | 10 | 35 |
| Genotypes (%) | 45 | 22 | 33 | 4 | 36 | 60 | 61 | 28 | 10 | 33 | 15 | 52 |

[1] HR, highly resistant; MR, moderately resistant; S, susceptible

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## Results and discussion

The response of the cultivars to the three pathogens that most affected yield (PVY, late blight and early blight) shows that there are very large genotypic differences (Table 1), as well as for tuber yield (Table 2). A significant proportion of the cultivars showed moderate or high levels of susceptabilty for all three pathogens (PVY 55%, late blight 96%, early blight 38%). In case of PVY, virtually all individual plants of PVY susceptible cultivars became infected during the three growing season. Contrary to PVY which is transmitted by aphids, the reaction of plants to fungal diseases and the rate of infestation depends to a great extent on environmental factors (*e.g.*, amount and distribution of precipitation, temperature, etc.). The year 2021 was basically a dry, warm season that was not advantageous to late blight infection. However, precipitation in the third part of the growing season resulted in significant infestation, especially for late maturing cultivars. This season, however, was especially favorable for the development of early blight. Nevertheless, only 10% of the varieties showed a severe infection and 28% showed only moderate symptoms.

Overall, a number of characteristics need to be considered simultaneously when selecting the most suitable varieties for organic production (Table 3). Depending on growing conditions, the response to biotic and abiotic stress factors of the varieties is much more important than in conventional farming. In the agroecological environment of Central Europe, resistance to PVY plays a key role in this respect, in addition to the response to late blight.

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**Table 2** Variation in tuber yield in the ECOBREED potato working collection.

| | Tuber yield (t·ha$^{-1}$) | | | Marketable yield (%) | | |
|---|---|---|---|---|---|---|
| Score | > 10 | 10-20 | < 20 | > 60 | 60-45 | < 45 |
| Genotypes (*n*) | 20 | 40 | 7 | 8 | 19 | 40 |
| Genotypes (%) | 30 | 60 | 10 | 12 | 28 | 60 |

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**Table 3** Combination of favorable traits in the best performing varieties of the ECOBREED potato working collection (disease reaction HR = highly resistant).

| Positive traits (*n*) | Cultivar | PVY HR | Late blight HR | Early blight HR | Marketable yield >60% | Total yield >20 t·ha$^{-1}$ | Marketable yield (t·ha$^{-1}$) | Maturity class |
|---|---|---|---|---|---|---|---|---|
| 5 | Damaris | + | + | + | + | + | 18.3 | medium |
| 5 | 12-LHI-6 | + | + | + | + | + | 8.3 | late |
| 5 | White Lady | + | + | + | + | + | 8.3 | late |
| 4 | Botond | + | + | + | + | | 8.9 | early |
| 4 | Michalia | + | + | + | | + | 7.5 | early |
| 4 | Lilly | + | + | + | + | | 6.1 | medium |
| 4 | Omega | + | + | + | + | | 5.0 | early |
| 3 | Fidelia | + | | + | | + | 13.0 | medium |
| 3 | Otolia | + | | + | | + | 11.1 | late |
| 3 | Caprice | + | + | + | | | 9.7 | medium |
| 3 | Agria | + | + | + | | | 8.2 | late |
| 3 | Balatoni | + | + | + | | | 7.9 | early |
| 3 | Basa | + | + | + | | | 6.5 | late |
| 3 | Goldmarie | + | + | + | | | 6.3 | medium |
| 3 | Bzura | + | + | + | | | 3.4 | late |
| 2 | KIS Slavnik | + | | | | + | 7.8 | early |

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# Marker-assisted and effector selection of potato genotypes with quantitative resistance to late blight

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## Abstract

The oomycete *Phytophthora infestans* is the causal agent of late blight and a major cause of enormous yield losses in potato production. With the increase in genetic diversity through sexual recombination following the worldwide spread of mating type A2 and the emergence of fungicide-resistant genotypes, the development of new potato varieties with high resistance to late blight has become a high priority for potato breeding programs. Multiple stacking of resistance (*R*) genes and QTL conferring quantitative resistance led to the development of durable late blight resistant cultivars such as 'Sarpo Mira', which was developed in the 1990s. The high resistance of 'Sarpo Mira' to late blight is conferred by four qualitative resistance genes (*R3a, R3b, R4, and Rpi-Smira1*) and one quantitative gene (*Rpi-Smira2/R8*). It was hypothesized that the latter is the gene that contributes the most in 'Sarpo Mira'. To determine the contribution of *Rpi-Smira2/R8* to late blight resistance, potato genotypes containing only the *R8* gene were developed from progeny of crosses between 'Sarpo Mira' and late blight susceptible cultivars.

Five susceptible potato cultivars ('Rioja', 'Lusa', 'Colomba', 'Bikini', and 'Sylvana') were crossed with 'Sarpo Mira' at the Agricultural Institute of Slovenia. A total of 1420 collected true seeds were sown in the greenhouse (growth conditions: 16 h/8 h day/night, temperature 21 ± 3°C), of which 1213 potato seedlings germinated successfully. Selection of *R8* potato genotypes began with marker-assisted selection (MAS), using genetic markers for the *R3a, R3b*, and *Rpi-Smira1* genes to verify the absence of these quantitative resistance genes. After DNA extraction from 4-week-old plants, MAS was performed stepwise, starting with the *R3b* gene. Only 186 samples were subjected to negative selection for genetic markers for genes *R3a* and *Rpi-Smira1*, resulting in 104 samples. The genetic marker for gene *Rpi-Smira2/R8* was used for the final positive selection, with 36 samples testing positive for this gene.

Since the genetic marker for the *R4* gene was not available, effector agroinfiltration was used as an alternative approach to discriminate between *R8* genotypes and genotypes containing both *R8* and *R4* genes from the collection of 36 samples. A suspension of *Agrobacterium tumefaciens* containing a vector with the *Avr4* gene was first infiltrated onto whole plants. However, due to the robustness and thickness of potato leaves, additional pressure had to be applied to the leaves with a syringe, resulting in severe tissue damage. To avoid this, agroinfiltration was performed on detached leaves of all 36 plants tested. In addition, all detached leaves tested were first nicked on the abaxial side with a sterile needle under a stereomicroscope at several locations to damage the surface of the leaf tissue but not puncture it. This allowed easier and more effective infiltration of the *A. tumefaciens* suspension. After three days, leaves were checked for the presence of a hypersensitive reaction indicating the presence of the *R4* gene in the plant tissue. Of the 36 potato genotypes tested, ten genotypes showed no signs of hypersensitive reaction, thus were free of *R4* gene, but contained *Rpi-Smira2/R8* gene. Two genotypes were progeny from the cross between 'Rioja' and 'Sarpo Mira', one genotype was a progeny from the cross with 'Lusa', three genotypes were progeny from the cross with 'Colomba', and four genotypes were progeny from the cross with 'Sylvana'. None of the progeny were from the cross between 'Bikini' and 'Sarpo Mira'.

In this study, a combination of MAS and the modified effector *Avr4* agroinfiltration was used to successfully obtain potato genotypes containing only the *Rpi-Smira2/R8* gene. These *R8* genotypes were transferred to *in vitro* culture and inoculated with different *P. infestans* isolates to determine their resistance levels. Preliminary studies show that some *R8* genotypes have significantly higher resistance to late blight than their complementary susceptible parent cultivar. In addition, after inoculation with aggressive *P. infestans* isolates, one *R8* genotype showed similar resistance to late blight as the resistant cultivar 'Sarpo Mira'.

## Keywords

Agroinfiltration · genetic markers · *Phytophthora infestans* · *Rpi-Smira2/R8* gene · *Solanum tuberosum*

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# Physiological response to drought stress in two potato (*Solanum tuberosum* L.) cultivars under greenhouse conditions

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## Abstract

The potato crop is of great economic value and is considered as a drought-sensitive crop. Its production in Europe has been increasingly affected by droughts over the last 20 years and these will become even more frequent in the future due to climate change. Breeding programmes should therefore include selection for drought tolerance, which has not yet been fully exploited. There is an even greater need for drought-tolerant potato cultivars for low-input and environmentally sustainable agricultural practices, such as organic production. The aim of this preliminary study is to evaluate the response of two Slovenian cultivars to drought stress under greenhouse conditions by monitoring physiological parameters. Obtaining new information on the two cultivars and identifying drought tolerance traits will lead to improvement of selection methods in the organic breeding programme of the Agricultural Institute of Slovenia, so that well adapted drought tolerant potato cultivars can be produced in the future.

We conducted the greenhouse experiment from February to April 2021 at the Agricultural Institute of Slovenia (Ljubljana, Slovenia) in a chamber with controlled environmental conditions (T = 21°C/15°C, RH = 60 %, photoperiod 14 h light/10 h dark). We used two potato cultivars for the experiment: KIS Savinja and KIS Vipava. For each cultivar, we planted 12 tubers in 5-litre pots filled with *Sphagnum* peat substrate (Potgrond H, Klassmann). At the beginning of the treatment, about 8 weeks after planting, each pot was watered to field capacity. Terminal water stress was then imposed by leaving the pots with the plants without additional irrigation until the end of the experiment. We made daily physiological measurements for 17 consecutive days. Each day, we randomly selected 4 plants from each of the two cultivar groups (8 plants in total) and measured the midday leaf water potential (WP) using the Scholander pressure chamber and the maximum quantum efficiency of PSII photochemistry (Fv/Fm) using the PAM-2100 fluorometer (H. Walz GmbH, Effeltrich, Germany). We also measured their leaf physiological parameters using the LI-COR 6400 XT (LI-COR Biosciences GmbH, Bad Homburg, Germany) with the leaf chamber fluorometer connected to it. We measured seven main parameters: carbon assimilation rate (Photo), stomatal conductance (Cond), intracellular carbon concentration ($C_i$), maximum efficiency of PSII photochemistry under light conditions (Fv′/Fm′), PSII efficiency (PhiPS2), electron transport rate (ETR), transpiration (Trmmol). From Photo and Cond we calculated the water use efficiency (WUE). Measurements were grouped by cultivar and two WP ranges: [-0.1, -0.4 MPa] with no visible signs of stress on the plants, defined as 'no stress' (NS) and [-0.6, -0.9 MPa], where wilting and yellowing of leaves occurred, defined as 'drought' (D). Within each range, the difference between cultivars was analysed using the Kruskal-Wallis one-way test of variance using R (R Foundation) and RStudio (Boston, MA). Effect sizes were estimated using eta-squared estimation (rstatix 0.7.0 package). In addition, we modelled the progression of drought by the decrease in WP in relation to the day of the experiment. Several candidate models were made with the help of the drc package. All models were tested with the AICc function from the rstatix package, the model with the lowest AICc (Brain-Counsen hormesis model) was selected to fit the data.

Of the eight variables tested, six showed a statistically significant difference between cultivars. WUE (NS: $p = 0.029$; $\eta^2 = 0.18$, D: $p = 0.004$; $\eta^2 = 0.14$), Ci (NS: $p = 0.029$; $\eta^2 = 0.18$; D: $p = 0.003$; $\eta^2 = 0.14$) and Fv′/Fm′ (NS: $p = 0.031$; $\eta^2 = 0.15$, D: $p = 0.002$; $\eta^2 = 0.15$) were statistically significant under drought stress and no stress, Fv/Fm ($p < 0.001$; $\eta^2 = 0.21$), PhiPS2 ($p < 0.001$; $\eta^2 = 0.26$) and ETR ($p < 0.001$; $\eta^2 = 0.26$)) were statistically significant only under drought stress. Overall, the difference in the photochemical system performance between two cultivars appears to be large ($\eta^2 \geq 0.14$). 'KIS Savinja' seems to be better at maintaining its photochemical function under drought conditions, except in maintaining concentrations of Ci (Fig. 1). 'KIS Savinja' also exhibited a different wilting pattern with its turgor loss starting at the base of the plant and moving upward. Single plants of 'KIS Vipava' wilted uniformly along the entire stem axis. Further testing is needed to determine exactly how the cultivars differ for other physiological responses, particularly water loss (transpiration, stomatal conductance).

## Keywords

Drought tolerance · plant physiology · potato varieties · sustainable agriculture · water stress

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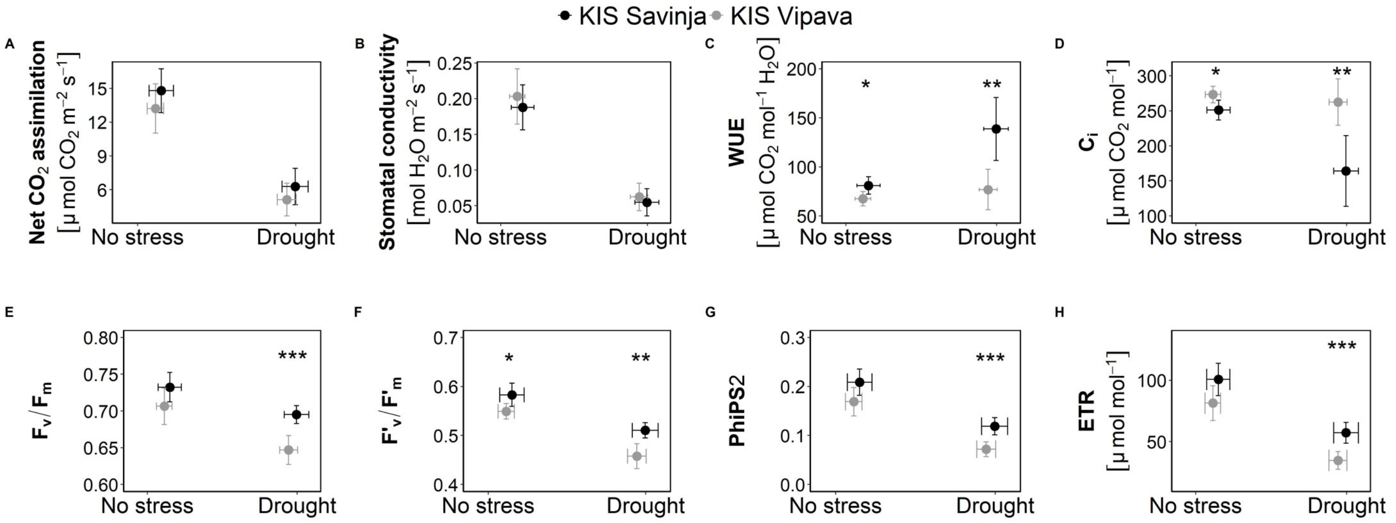

**Figure 1** Changes in physiological parameters under drought stress in potato cultivars 'KIS Savinja' and 'KIS Vipava'. The data are distributed according to the water potential range: **a** net CO2 assimilation; **b** stomatal conductivity; **c** water use efficiency; **d** intracellular carbon concentration; **e** maximum quantum efficiency of PSII photochemistry; **f** maximum quantum efficiency of PSII photochemistry under light conditions; **g** PSII efficiency; **h** electron transport rate

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# Phenotypic characterisation of ECOBREED buckwheat genetic resources

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## Abstract

Buckwheat is a pseudocereal with important nutritional qualities and great potential for broad consumption. Two species of buckwheat are among the most extensively produced and consumed around the world: common buckwheat (*Fagopyrum esculentum* Moench) and Tartary buckwheat (*F. tataricum* (L.) Gaertn.). Over recent decades, many buckwheat accessions have been collected in different parts of Europe and are stored in national gene banks, although the characterisation data that are needed for future use and breeding are still lacking and/or are not easily accessible. The information about individual accessions, and particularly those conserved in *ex situ* collections, is often poor, reducing the frequency and efficiency of its use, and likewise, the ultimate benefits this information can provide.

In the present study, common and Tartary buckwheat germplasm was evaluated using different descriptors. Phenotypic descriptors adopted by the International Union for the Protection of New Varieties of Plants (UPOV) and the International Board for Plant Genetic Resources (IBPGR) were applied. Such characterisation could provide useful information for plant breeders for the breeding of new varieties and for further studies of the morpho-agronomic traits of *Fagopyrum* spp. The present collection comprises a total of 248 accessions of which 196 belong to common and 52 to Tartary buckwheat. The plant material was cultivated in pots and characterised using 40 numeric (quantitative) and descriptive (qualitative) descriptors for *Fagopyrum* spp. based on germination, growth habit, plants, stems, inflorescences, leaves, etc.

The germination descriptors comprised germination (%), cotyledon/seedling leaf colour, cotyledon anthocyanin coloration, and germination period (days). The plant descriptors comprised growth and branch shoot habit, plant height (cm), plant branching, lodging susceptibility, time of beginning of flowering, days to flowering, days to maturity, and time of maturity. The stem descriptors comprised stem colour, diameter, number of nodes, and anthocyanin coloration. The inflorescence descriptors were for anthocyanin coloration of the bud, compactness of inflorescence, branched inflorescence and colour of inflorescence stalk. The cyme descriptors comprised length of cyme (cm), number of flower clusters per cyme and number of cyme per plant. The flower descriptors comprised flower colour, flower morphology and colour of petals. The leaf descriptors comprised leaf colour, leaf margin colour, leaf vein colour, intensity of leaf colour, leaf blade and base shape, leaf number, leaf blade length (cm) and width (cm), petiole length (cm) and petiole colour.

All characteristics measured quantitatively or assessed qualitatively showed a wide range of variation among the evaluated *Fagopyrum* accessions. As a further step, analysis of this diversity using molecular approaches is recommended, to provide more information on the overall genetic diversity, and potentially on the genes responsible for the specific agro-morphological traits in this *Fagopyrum* spp. germplasm.

## Keywords

Trait descriptor · *Fagopyrum* · germplasm collection · phenotyping

## Acknowledgements

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# Preparation of buckwheat DNA extracts for further marker-assisted selection activities

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## Abstract

Extraction of high quality DNA is crucial for any genetic analysis. However, it is difficult to extract it from problematic plant tissues rich in secondary metabolites and other compounds whose content varies among species/varieties.  The aim of the study was to optimize the DNA extraction protocol for marker-assisted selection (MAS) of buckwheat (*Fagopyrum esculentum, F. tataricum*), including molecular genotyping with functional DNA markers and/or NGS-based (Next Generation Sequencing) methods. In addition, the possibility and usefulness of extracted DNA as freeze-dried was of great importance for shipping samples worldwide.

Three different extraction methods were used according to the manufacturer's instructions for both types of DNA from the first well-developed leaf tissue: i) magnetic extraction with the automated MagMax nucleic acid isolation robot (Thermo Fisher Scientific) using the BioSprint 96 Plant Kit (Qiagen); ii) the DNeasy Plant Mini Kit (Qiagen); iii) the DNeasy Plant Pro Kit (Qiagen) with and without the addition of PS buffer (for problematic samples). DNA extraction with the MagMax kit was unsuccessful, while we did not obtain DNA from any of the buckwheat species. For the other two kits, extractions were successful (Fig. 1) for both buckwheat species based on nanodrop measurements. For common buckwheat, yields ranged from 2.4 ng µL$^{-1}$ to 33.7 ng µL$^{-1}$; 260/280 ratios ranged from 1.49 to 2.46; and 260/230 ratios ranged from 0.45 to 3.3. For Tartary buckwheat, DNA yields ranged from 3.7 ng µL$^{-1}$ to 22.3 ng µL$^{-1}$; the 260/280 ratio ranged from 1.14 to 2.45 as the 250/230 ratio ranged from 0.68 to 2.04.

The purity, quality and quantity of these DNA extracts would be sufficient for in-house performance of MAS. If the freeze-drying process of buckwheat DNA samples for long distance shipping is required to perform NGS-based MAS applications, these methods are less suitable. According to our results, we could suggest that the optimized CTAB-based method is more suitable before the DNA is prepared as freeze-dried powder. In addition, the time and cost of an extraction method should not be ignored, especially when a large number of samples are involved.

## Keywords

DNA quality · *Fagopyrum* sp. · genotyping · extraction methods

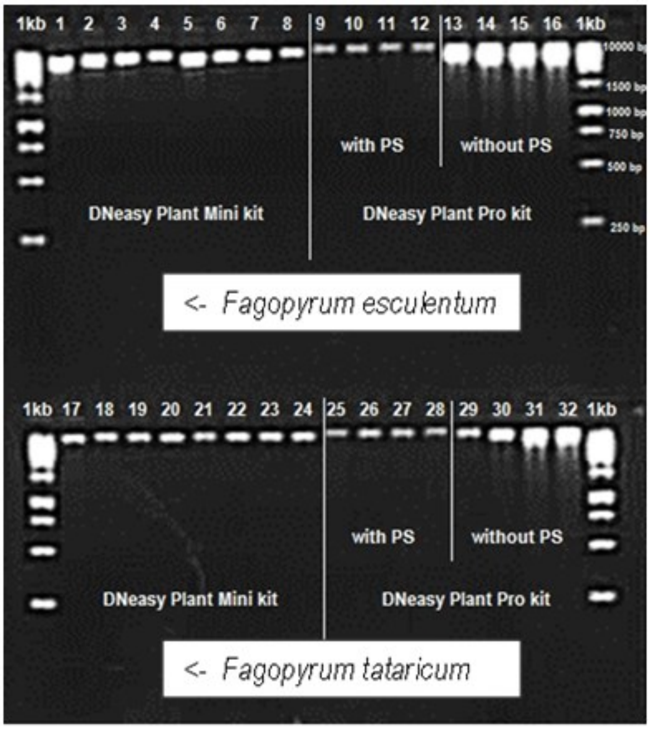

**Figure 1** DNA profiles of common and Tartary buckwhat DNA resulting from different extraction methods

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# Phenolic compounds in buckwheat: Road to agroecology

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## Abstract

Common buckwheat (*Fagopyrum esculentum*) contains secondary metabolites with significant plant growth inhibitory activity, which can be exploited for weed management. The study of the allelopathic activity of those metabolites may allow the selection of buckwheat varieties with high allelopathy suitable for organic farming. The present study aimed to (i) evaluate the allelopathic activity of buckwheat accession 'Gema' against germination of target weeds, (ii) to identify and to quantify the secondary metabolites (*i.e.*, phenolic acids and flavonoids), and (iii) to evaluate the impact on photosynthetic pigments of weeds.

The allelopathic effects of 'Gema' were evaluated on a monocot weed, *i.e.*, annual ryegrass (*Lolium rigidum* L.), and a dicot weed, *i.e.*, common purslane (*Portulaca oleracea* L.), under controlled laboratory conditions. Pre-germinated seedlings of 'Gema' were grown for one week in one half of plastic trays containing perlite. Ten seeds of either *P. oleracea* or *L. rigidum* were planted in the other half of the same plastic tray for one more week using the equal-compartment (EC) method. Germination, biomass, and seedling growth and photosynthetic pigment contents of both weeds were measured. The polyphenol contents present in shoots, roots and rhizosphere water of 'Gema' were quantified using LC-MS.

The results showed that 'Gema' affected germination and growth of *L. rigidum*, suggesting that the monocot weed did not represent a problem for 'Gema'. In case of *P. oleracea*, the presence of 'Gema' induced a decrease in germination but an increase in root length. After 10-days co-culture with 'Gema', both *P. oleracea* and *L. rigidum* showed a disproportionate increase in violaxanthin (+437% and 544%, respectively) and a decrease in neoxanthin (-98% and -96%, respectively) (Table 1). Neoxanthin is the last product in the carotenoid biosynthetic pathway, but is also a precursor in the biosynthesis of ABA. Violaxanthin is transformed into neoxanthin by the neoxanthin synthase, so it is assumed that some factor is modifying the activity of this enzyme that prevents the transformation into neoxanthin in both weeds. Since the dominant ABA formation pathway in stressful situations is via neoxanthin, these weeds may be compromised in their response to stress by ABA deficiency. Due to this situation, *P. oleracea* reacted by increasing the production of β-carotene (+282%) and lutein (+53%) as a response to 'Gema' co-cultivation, which correlates with the growth and germination data. However, *L. rigidum* was not able to orchestrate this stress response, being more defenseless in front of this buckwheat variety, which is correlated with the growth decrease we observed on the weed.

## Keywords

Allelopathy · *Fagopyrum escultentum* · photosynthetic pigment · secondary metabolite

**Table 1** Concentrations of photosynthetic pigments in the leaves of *Portulaca oleracea* and *Lolium rigidum* when grown alone (control) or in association with buckwheat variety 'Gema'. Data are means of three replicates; +/- indicates an increase and or decrease in %, respectively, in the presence of buckwheat; Chl *a*, chlorophyll a; Chl *b*, chlorophyll b; β-Car, β-carotene; Lut, lutein; Neo, neoxanthin; Vio, violaxanthin; pigments content is expressed in relation to chlorophyll calibration curve (Chl *a* and Chl *b*) or to carotene calibration curve (α- and β-carotene, and xanthophylls).

| Treatment | Chl *a* | +/- | Chl *b* | +/- | β-Car | +/- | Lut | +/- | Neo | +/- | Vio | +/- |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *P. oleracea* | | | | | | | | | | | | |
| Control | 3106 | | 1203 | | 468 | | 2915 | | 315.9 | | 380 | |
| Gema | 6232 | +101 | 2024 | +68 | 1785 | +282 | 4463 | +53 | 6.8 | -98 | 2040 | +437 |
| *L. rigidum* | | | | | | | | | | | | |
| Control | 6302 | | 4011 | | 1216 | | 4376 | | 812.0 | | 257 | |
| Gema | 9389 | +49 | 2738 | -32 | 1651 | +36 | 4212 | -4 | 35.0 | -96 | 1653 | +544 |

Vieites Álvarez Y, Sánchez-Moreiras AM, Reigosa Roger MJ, Hussain MI (2022) Phenolic compounds in buckwheat: Road to agroecology. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 72. Jahrestagung 2021, 22-24 November, Raumberg-Gumpenstein, pp 105-106. University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-96-1

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# Phenotypic plasticity, yield stability and signature of stable isotopes of carbon and nitrogen in safflower under saline environment

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## Abstract

Salinity is one of the major factors contributing to land degradation, disturbance of soil biology, structure that leads to unproductive land with low crop yield potential especially in arid and semiarid regions. Appropriate crops with sufficient stress tolerance potential and non-conventional water resources should be managed in a sustainable way to bring these marginal lands under cultivation for future food security. The goal of the present study was to evaluate the salinity tolerance (0, 7, and 14 $dS\cdot m^{-1}$) of six safflower genotypes (*i.e.*, PI 167390, PI 239707, PI 248836, PI 250714, PI 253385, and PI 253387) that can be adapted to the hyper arid climate of the United Arab Emirates (UAE). Salinity is a major abiotic stressor that reduces the growth, yield, and quality of major cereals, feeds, and oilseeds worldwide. Meanwhile, crop management practices and selection of suitable crop genotypes can play a critical role in adapting and improving conditions in saline and water-scarce environments, and are the best options for salinity mitigation. In addition, the selection of appropriate agro-physiological and biochemical characteristics should be prioritized to understand the mechanisms involved in resistance to abiotic stresses. Safflower (*Carthamus tinctorius* L.) is a vital oil seed crop, grown in arid and semiarid regions. It constitutes 35-50% oil, minerals (Zn, Cu, Mn and Fe), vitamins (thiamine and β-carotene), α-, β- and γ-tocopherols and bioactive compounds.

Six safflower genotypes were sown during the first week of November 2013 by hand in the field plots ($2\times4$ m = 8 $m^2$), in a split-plot design with three replications. Salinity levels (*i.e.*, S1: 0 = control; S2: 7 $dS\cdot m^{-1}$; and S3: 14 $dS\cdot m^{-1}$) was the main factor and genotypes were the sub factors. There were four rows (each row of 4 m in length) in each plot with a row spacing of 0.5 m between them. The salinity level in the irrigation water was kept the same and checked bi-weekly. The field experiment was equipped with a drip irrigation system (4 $L\cdot hr^{-1}$ flow rate), 0.5 m distance between rows and 0.25 m between drippers. Irrigation was applied at rates equivalent to $ET_0$ plus 10% for leaching requirements. After harvest, all plots were irrigated at $ET_0$ plus 25% for additional leaching. Physiological traits such as plant dry biomass (PDM), number of branches (BN), number of capitula (CN), seed yield (SY), stable isotope composition of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$), intercellular $CO_2$ concentration from inside to ambient air ($Ci/Ca$); intrinsic water use efficiency (iWUE); carbon (C%) and nitrogen (N %), and harvest index (HI) were evaluated as indicators of the functional performance of safflower genotypes under salt stress.

The salinity levels (7 and 14 $dS\cdot m^{-1}$), reduced the number of capitula and this reduction was in the range of 24-44%, respectively. Higher seed yield was obtained in control plots while yield significantly decreased at 14 $dS\cdot m^{-1}$. Highest seed yield was obtained for PI 248836, and lowest for PI 239707. Harvest index (HI) was reduced after salt water treatments in the range of 10.2-32.6% at 7 and 14 $dS\cdot m^{-1}$, respectively (Table 1). Harvest index (HI) greatly varied among the safflower genotypes. PI 248836 exhibited the highest HI, and PI 239707 the lowest, similar to the impact of salinity on seed yield. $\Delta^{13}C$ varied significantly ($p > 0.05$). PI 239707 demonstrated lowest Δ value (19.6‰), while PI 248836 showed the highest Δ (25.6‰). The salt sensitive safflower genotypes exhibited the lowest Δ values (19.6 - 22.2‰) but relatively high iWUE. PI 253387 and PI 239707 possess lower grain yield but higher i-WUE. The second grade included genotypes with Δ values slightly higher (2.8 to 24.4‰) and seed yield was quite variable, ranging from 2.4 to 2.7 $t\cdot ha^{-1}$. In the third grade, genotypes had the highest Δ values, ranging from 25.2 to 25.6‰ and also had highest seed yield with a range of 3.1 to 3.6 $t\cdot ha^{-1}$. PI 167390 showed higher foliar N% values than all other genotypes. The results indicated that salinity significantly affected seed yield at all levels and varied significantly among genotypes. The BN, PDM, CN, $\delta^{13}C$ attributes showed clear differentiation between tolerant and susceptible genotypes. The $\delta^{13}C$ indicate that tolerant genotypes suffer less from salt stress that may be due to better rooting. Tolerant genotypes showed lower iWUE values but possess higher yield. Genotypes PI 248836 and PI 167390 proved to be salt tolerant, showing stable and higher seed and biomass yield. Genotypes that produce higher yield under control conditions were still best even under salt stress conditions. Although salinity reduced crop yield, some tolerant genotypes demonstrate the potential to use saline water and marginal lands for cultivation of oilseed crops.

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**Table 1** Genotype and treatment effects on biomass, agro-physiological and yield traits and carbon isotope composition of six safflower genotypes grown under different water salinity levels.

| | BN[1] | PDM | CN | SY | HI | *Ci*/*Ca* | $\delta^{13}C$ | $\Delta^{13}C$ | iWUE |
|---|---|---|---|---|---|---|---|---|---|
| Genotypes | | | | | | | | | |
| PI 248836 (tolerant) | 42.2b | 9.7a | 598b | 3.6a | 36.7a | 0.94a | -32.73a | 25.6a | 1.3d |
| PI 167390 (tolerant) | 50.0a | 10a | 727.1a | 3.1a | 30.4bc | 0.92a | -32.38a | 25.2a | 1.7d |
| PI 253387 (MT) | 29.9d | 8.9b | 448.4d | 2.7b | 30.4bc | 0.82b | -32.19c | 22.8c | 3.9b |
| PI 250714 (MT) | 37.3c | 7.5b | 546.2c | 2.4bc | 31.6b | 0.88b | -31.63b | 24.4b | 2.5c |
| PI 253385 (sensitive) | 29.8d | 6.8c | 362.4f | 2.3c | 31.3b | 0.78c | -29.55d | 22.2c | 4.6b |
| PI 239707 (sensitive) | 30.9d | 8.6b | 379.7e | 2.2c | 25.4d | 0.67d | -27.11e | 19.6d | 7.1a |
| Treatment[2] | | | | | | | | | |
| S1-0 (Control) | 43.6a | 9.8a | 599.3a | 3.5a | 35.8a | 0.800b | -31.70a | 24.5a | 2.3c |
| S2-7 dS $m^{-1}$ | 34.7b | 8.3b | 499.5b | 2.6b | 31.9b | 0.863a | -30.50b | 23.2b | 3.6b |
| S3-14 dS $m^{-1}$ | 31.6c | 7.7c | 432.1c | 1.9c | 25.1c | 0.786c | -29.40c | 22.1c | 4.7a |
| Level of significance | | | | | | | | | |
| Genotype (G) | .00 | .00 | .00 | .00 | .18 | .00 | .00 | .00 | .00 |
| Treatment (T) | .00 | .00 | .00 | .00 | .00 | .00 | .00 | .00 | .00 |
| G x T interaction | ns | ** | ns | ns | ns | ns | ns | ns | ns |

[1] BN, branches ($n\cdot m^{-2}$); PDM, plant dry biomass ($t\cdot ha^{-1}$); CN, capitula ($n\cdot m^{-2}$); SY, seed yield ($t\cdot ha^{-1}$); HI, harvest index (%); *Ci*/*Ca,* ratio of intercellular to ambient $CO_2$ concentration; $\delta^{13}C$, stable carbon isotope composition (‰); $\Delta^{13}C$, carbon isotope discrimination (‰) Means followed by different letters are significantly different ($p < 0.05$) according to Tukey's honestly significant difference (HSD) test; MT, medium tolerant.

[2] S1-0 (control treatment); S2-7 $dS\cdot m^{-1}$, medium salinity; S3-14 $dS\cdot m^{-1}$, high salinity; ns, not significant; G, genotype; T, treatment

**Keywords**

Carbon discrimination · *Carthamus tinctorius* · genotype evaluation · nitrogen discrimination · salinity · yield

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