73rd Conference

21-23 November 2022

New plant breeding techniques: myths, facts and reality

Results from the ECOBREED project

BOKU

SAATGUT AUSTRIA

SAATGUT AUSTRIA

Vereinigung der Pflanzenzüchter
und Saatgutkaufleute Österreichs

73rd Conference

21-23 November 2022
Raumberg-Gumpenstein

New plant breeding techniques: myths, facts and reality

co-hosted by

HBLFA Raumberg-Gumpenstein Landwirtschaft

BOKU IPZ Institute of Plant Breeding

Imprint

Proceedings of the 73rd Conference of the *Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs*, 21-23 November 2022, Raumberg-Gumpenstein, Irdning, Austria

Editor
Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
Wiener Str. 64
3100 St. Pölten
E-mail: office@saatgut-austria.at
URL: www.saatgut-austria.at

Technical editors
Dr. Anton Brandstetter, Manuela Geppner
Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs

A.o. Univ.Prof. Dr. Heinrich Grausgruber
Universität für Bodenkultur Wien

Responsible for the content
The authors

Photo front cover
Svenja Bomers, Austrian Agency for Health and Food Safety GmbH (AGES), Vienna
Testing of runner bean (Phaseolus coccineus) genetic resources for heat tolerance in the greenhouse. Currently grown varieties are susceptible to heat waves during flowering, resulting in significant yield losses due to pollen sterility and shedding of flowers and pods before seed set and filling. See contribution by Bomers *et al.* on page 71.

Publisher
Universität für Bodenkultur Wien
Department für Nutzpflanzenwissenschaften
Institut für Pflanzenzüchtung
Konrad Lorenz Str. 24
3430 Tulln an der Donau
E-mail: pflanzenzuechtung@boku.ac.at
URL: https://boku.ac.at/dnw/pz

ISBN-13: 978-3-900397-07-4
https://doi.org/10.5281/zenodo.7875597

Table of contents

73. Jahrestagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Nachruf: Michael Oberforster (1959-2022)

Klemens MECHTLER[1]

[1] Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH, Spargelfeldstr. 191, 1220 Wien, Austria

(✉) klemens.mechtler@ages.at

Dipl.-Ing. Michael Oberforster, Leiter der Fachgruppe Getreide und Sortenzulassungsadministration in der Österreichischen Agentur für Gesundheit und Ernährungssicherheit (AGES), ist am 25. Oktober 2022 unerwartet verstorben.

Michael Oberforster wurde am 26. Juli 1959 in Großraming, Oberösterreich, geboren. Nach der Absolvierung der Höheren Bundeslehranstalt in St. Florian und dem Studium der Landwirtschaft an der Universität für Bodenkultur Wien trat Michael Oberforster am 4. Jänner 1988 in die damalige Bundesanstalt für Pflanzenbau, eine Vorläuferinstitution der AGES, ein. Sein Hauptaufgabengebiet war von Beginn an die Prüfung und Bewertung von Getreidesorten im Zuge der Sortenzulassung. Die Gruppe der Getreidearten hat ihn nach eigener Aussage unter den landwirtschaftlichen Kulturen schon immer ganz besonders fasziniert und er fand in der Vielfalt und im österreichweiten Prüfnetz für die Getreidearten ein weites Tätigkeitsfeld vor. Michael Oberforster brachte für diese Aufgaben viel Talent, Begeisterung und einen ungeheuren Arbeitseinsatz mit. Auf dem Versuchsfeld galt er als genauer und aufmerksamer Beobachter, stets bemüht, den Dingen auf den Grund zu gehen für eine möglichst unverfälschte Beurteilung der Sortenkandidaten. Im Büro arbeitete er ständig an der Verbesserung der Prüfrichtlinien, der Versuchsdokumentation, an den Auswertungen und der Interpretation der Versuchsergebnisse, las Fachpublikationen oder verfasste selbst solche.

Im Oktober 1991 erfolgte die Ernennung zum Leiter der damaligen Abteilung für Getreidebau. Danach arbeitete er am bis heute gültigen Bewertungsschema '94 für die technologische Qualität von Winterweizensorten ebenso mit wie an der Etablierung der Sortenwertprüfungen auf Bioflächen bereits Mitte der 1990er Jahre. Sein Einsatz wurde im November 1998 mit der Verleihung des silbernen Ehrenzeichens für Verdienste um die Republik Österreich gewürdigt.

Bereits 2001 begann Michael Oberforster, erstmalig unter den Sortenprüfämtern in Europa, eine eigene Bio-Wertprüfung bei Winterweizen. Weitere wichtige Anpassungen in der Sortenwertprüfung wie die Einführung unterschiedlicher Produktionsintensitäten in der Versuchsführung, die Regionalisierung der Prüfsortimente (z.B. in die Winterweizen-Sortimente Ost und West), oder die Aufnahme neuer Prüfserien, wie z.B. für Winterhafer oder zuletzt für Winterbraugerste, waren ebenfalls seine Verdienste.

Ganz im Sinne der AGES waren ihm die Widerstandsfähigkeit der Sorten gegenüber Krankheiten und die Hochwertigkeit und Unbedenklichkeit der Ernteprodukte ein besonderes Anliegen. So führte er nach Mitarbeit an einem internationalen Forschungsprojekt bei Roggen die Anfälligkeit für Mutterkorn als relevantes Sortenmerkmal ein. Bei Weizen und Durum legte er besonderen Wert auf die geringe Belastung durch Ährenfusarium. Innerhalb von mehreren wissenschaftlichen Projekten mit deutschen Partnern forschte er an der Auswuchsfestigkeit von Weizen am Feld.

Michael Oberforster war seit 1997 Geschäftsführer der Sortenzulassungskommission und hat diesem Gremium in dieser Zeit etwa 2500 Sorten landwirtschaftlicher Arten für die Beurteilung ihres Landeskulturellen Wertes vorgestellt. Er bearbeitete zudem Fragestellungen zu Ertragsaufbau, Saatzeitoptimierung, Wirtschaftlichkeit von Fungizidanwendungen oder optimierten Einsatz von Stickstoffdüngern. In nationalen und internationalen Projekten forschte er zu Themen wie Ernährungssicherung, Klimawandel, Trockenstress, Biolandbau und Getreidequalität.

Kollege Oberforster galt als sehr belesen und als ein herausragender Experte mit umfangreichem Detailwissen. Er verfügte über

Mechtler K (2023) Nachruf: Michael Oberforster (1959-2022). In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 1-3. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

eine brillante Eloquenz im Vortrag und die Fähigkeit, fachlich komplexe Zusammenhänge, wie sie bei landwirtschaftlichen Versuchsfragen häufig auftreten, gut und verständlich darzulegen. Er war ein vielgefragter Referent bei Fachexkursionen, Feldtagen oder Erntegesprächen mit LandwirtInnen oder mit VertreterInnen des Agrarhandels und der verarbeitenden Industrie (z.B. Braugerstenkommittee, Sommerexkursion der Börse für landwirtschaftliche Produkte). Vielen LandwirtInnen stand er auch mit telefonischen und ausführlichen schriftlichen Auskünften zur Verfügung.

Michael Oberforster war ein steter Teilnehmer und oftmals Vortragender bei der Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute, der Jahresstagung der Arbeitsgemeinschaft für Lebensmittel-, Veterinär- und Agrarwesen oder der Gesellschaft für Pflanzenbauwissenschaften. Bei den jährlichen Treffen mit VertreterInnen der europäischen Sortenämter war er ein geschätzter Diskussionspartner für herausfordernde Fragestellungen im Sortenprüfwesen. Für sein umfangreiches Schaffen spricht nicht zuletzt auch seine Publikationsliste mit 630 Fachartikeln und 83 wissenschaftlichen Publikationen.

Michael Oberforster war ein verlässlicher Kollege, der jederzeit mit Rat und Tat zur Seite stand. Durch seinen Einsatz hat er das österreichische Sortenprüfwesen zukunftsorientiert mitgestaltet. Vorgaben, wie sie aus dem Green Deal-Programm, der Farm to Fork-Strategie oder den Diskussionen zur Neuausrichtung des europäischen Sorten- und Saatgutrechts abgeleitet werden können, sind dank seines Einsatzes für das österreichische Prüfsystem schon weitgehend umgesetzt.

Der österreichische Pflanzenbau verliert durch seinen Tod einen großen Fachmann mit unermüdlichem Einsatz zum Wohle der Landwirtschaft.

Wir werden unseren Kollegen und seine Leistungen in dankbarer Erinnerung behalten.

Beiträge von Michael Oberforster zur Züchtertagung

Oberforster M (2022) Austrian wheat varieties: Influence of plant height and dwarfing genes on susceptibility to Fusarium head blight. Bericht 72. Tagung 2021, pp 23-26.

Oberforster M, Flamm C, Freudenthaler P (2020) One hundred years of wheat varieties: breeding progress as measured by grain yield, agronomic traits and nitrogen efficiency. Bericht 70. Tagung 2019, pp 41-42.

Oberforster M, Marshall E (2019) Descriptive and recommended variety lists in European countries and their scales. Bericht 69. Tagung 2018, pp 59-71.

Oberforster M, Flamm C (2018) Six-row winter barley: comparison of hybrid and line cultivars. Bericht 68. Tagung 2017, pp 37-41.

Oberforster M, Kalmar E, Zechner E, Albrecht T, Hartl L (2016) Beziehung von Auswuchs, Keimungsindex und Fallzahlstabilität zur Fallzahl unter Belastungsbedingungen bei Winterweizen. Bericht 66. Tagung 2015, pp 39-42.

Oberforster M, Plank M (2015) Befall von Winterweizen, Sommerweizen und Triticale mit Gelbrost (*Puccinia striiformis*) in Österreich 2014 – Geänderte Sortenreaktion. Bericht 65. Tagung 2014, pp 93-99.

Oberforster M (2013) Probleme mit Auswinterung, Trockenstress, Virosen, Spätfrost und Zwiewuchs bei Weizen und Wintergerste im Jahr 2012 in Österreich. Bericht 63. Tagung 2012, pp 57-64.

Oberforster M, Neumayer A, Zechner E, Hartl L (2013) Einfluss von Reifeunterschieden auf das Auswuchsverhalten und die Fallzahl von Winterweizensorten. Bericht 63. Tagung 2012, pp 117-120.

Oberforster M, Ratzenböck A, Werteker M, Neumayer A, Zechner E, *et al*. (2012) Variabilität und Beziehungen von Samendormanz, Auswuchs und Fallzahl in einem Sortiment europäischer Winterweizen. Bericht 62. Tagung 2011, pp 61-68.

Oberforster M, Werteker M (2011) Inverse und nicht inverse Beziehungen von Kornertrag und Qualität im österreichischen Sortenspektrum von Weizen, Gerste und Roggen. Bericht 61. Tagung 2010, pp 9-17.

Oberforster M, Plank M, Bedlan G (2010) Phänotypische Charakterisierung der Anfälligkeit von Weizensorten gegenüber Schwarzrost (*Puccinia graminis* f. sp. *tritici*) in Österreich. Bericht 60. Tagung 2009, pp 183-186.

Oberforster M, Schulmeister K, Kainz W (2009) Wirkung langjähriger Erhaltungszüchtung auf Anbaueigenschaften, Ertrag und Qualität der Roggensorte EHO-Kurz. Bericht 59. Tagung 2008, pp 125-128.

Oberforster M, Köhldorfer R (2008) Genetische und umweltbedingte Variation des Stärkegehaltes von Weizen und Triticale in Hinblick auf die Bioethanolerzeugung. Bericht 58. Tagung 2007 pp 105-109.

Oberforster M, Werteker M (2006) Auswuchsneigung und Fallzahl als Sorteneigenschaft von Winterweizen, Roggen, Triticale und Winterdurum. Bericht 56. Tagung 2005, pp 103-112.

Oberforster M, Werteker M (1995) Das neue österreichische Bewertungsschema für Qualität von Weizensorten und seine Auswirkungen auf das Sortenspektrum. Bericht 45. Arbeitstagung 1994, pp 9-14.

Ausgewählte wissenschaftliche Publikationen

Albrecht T, Oberforster M, Kempf H, Ramgraber L, Schacht J, *et al*. (2015) Genome-wide association mapping of preharvest sprouting resistance in a diversity panel of European winter wheats. J Appl Genet 56:277-285. DOI: 10.1007/s13353-015-0286-5

Alexandrov V, Eitzinger J, Cajic V, Oberforster M (2002) Potential impact of climate change on selected agricultural crops in northeastern Austria. Global Change Biol 8:372-389. DOI: 10.1046/j.1354-1013.2002.00484.x

Börner A, Nagel M, (…), Oberforster M, Albrecht T, Mohler V (2018) QTL analysis of falling number and seed longevity in wheat (*Triticum aestivum* L.). J Appl Genet 59:35-42. DOI: 10.1007/s13353-017-0422-5

Gröger S, Oberforster M, Werteker M, Grausgruber H, Lelley T (1997) HMW glutenin subunit composition and bread making quality of Austrian grown wheats. Cereal Res Commun 25:955-962. DOI: 10.1007/BF03543902

Knapp S, Brabant C, Oberforster M, Grausgruber H, Hiltbrunner J (2017) Quality traits in winter wheat: comparison of stability parameters and correlations between traits regarding their stability. J Cereal Sci 77:186-193. DOI:10.1016/j.jcs.2017.08.011

Kodisch A, Schmiedchen B, Eifler J, (…), Oberforster M, Miedaner T (2022) Maternal differences for the reaction to ergot in unfertilized hybrid rye (*Secale cereale*). Eur J Plant Pathol 163:181-191. DOI: 10.1007/s10658-022-02467-0

Kodisch A, Wilde P, (…), Oberforster M, (…) Nistrup Jørgensen L, Miedaner T (2020) Ergot infection in winter rye hybrids shows differential contribution of male and female genotypes and environment. Euphytica 216:65. DOI: 10.1007/s10681-020-02600-2

Manschadi AM, Palka M, Fuchs W, (…), Oberforster M, Soltani A (2022) Performance of the SSM-iCrop model for predicting growth and nitrogen dynamics in winter wheat. Eur J Agron 135:126487. DOI: 10.1016/j.eja.2022.126487

Olesen JE, Børgesen CD, Elsgaard L, (…), Oberforster M, van der Fels-Klerx HJ (2012) Changes in time of sowing, flowering and maturity of cereals in Europe under climate change. Food Add Cont Pt A 29:1527-1543. DOI: 10.1080/19440049.2012.712060

Schils R, Olesen JE, Kersebaum K-C, Rijk B, Oberforster M, *et al*. (2018) Cereal yield gaps across Europe. Eur J Agron 101:109-120. DOI: 10.1016/j.eja.2018.09.003

Spiegel H, Sager M, Oberforster M, Mechtler K, Stüger HP, *et al*. (2009) Nutritionally relevant elements in staple foods: influence of arable site versus choice of variety. Env Geochem Health 31:549-560. DOI: 10.1007/s10653-009-9254-5

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Nachruf: Wilhelm Haupt (1934-2022)

Anton BRANDSTETTER[1]

[1] Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs, Wiener Str. 64, 3100 Sankt Pölten, Austria

(✉) anton.brandstetter@lk-noe.at

Prof. Dipl.-Ing. Wilhelm Haupt, geboren am 9. August 1934, ist am 15. Dezember 2022 verstorben.

Wilhelm Haupt leitete von 1979 bis 1999 als Pflanzenbaudirektor die Abteilung Pflanzenproduktion der Niederösterreichischen Landes-Landwirtschaftskammer. Seit 1973 war Wilhelm Haupt stellvertretender Geschäftsführer und von 1975 bis 2000 Geschäftsführer der Vereinigung der Pflanzenzüchter Österreichs. Diese Vereinigung war eine der Vorgängerorganisationen der heutigen Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Saatgut Austria).

In seiner Funktion als Geschäftsführer war er gemeinsam mit Wissenschaftlern der Universität für Bodenkultur Wien, der damaligen Bundesanstalt für alpenländische Landwirtschaft Gumpenstein (heute HBLFA Raumberg-Gumpenstein) und der damaligen Bundesanstalt für Pflanzenbau und Samenprüfung (heute AGES) für die Durchführung der jährlichen Pflanzenzüchtertagung in Raumberg-Gumpenstein verantwortlich. Dort wurden die aktuellen Fragen der Pflanzenzüchtung und Saatgutproduktion im Austausch zwischen Wissenschaft und Praxis behandelt.

Wilhelm Haupt hat sich für den Erhalt der österreichischen Pflanzenzüchtung eingesetzt und war Fürsprecher für die österreichische Saatgutproduktion. Er hat sich auch innerhalb der Landwirtschaft für einen hohen Saatgutwechsel eingesetzt, wodurch der Fortbestand einer österreichischen Züchtung ermöglicht wurde.

Er hat vor und nach dem EU-Beitritt Österreichs alle Aktivitäten unterstützt, die der Saatgutwirtschaft in Österreich wirtschaftliche Rahmenbedingungen ermöglichten und dadurch entsprechende Investitionen besonders in der Produktion von Qualitätssaatgut ausgelöst. Als Fachmann war sein Ziel, den Landwirten in Niederösterreich die besten Sorten bei bester Saatgutqualität zugänglich zu machen.

Die besten Sorten, welche von offizieller Stelle unter österreichischen Produktionsbedingungen getestet wurden und der Saatgutwechsel, waren - neben der Schulung und Beratung und der innovativen Entwicklung der Landwirte in Ihren Betrieben - wesentliche Gründe, dass die Hektarerträge in Österreich über Jahre hinweg kontinuierlich gestiegen sind. Das war auch die damalige Grundlage der neu entstandenen Qualitätsweizen und Kontraktweizenaktionen, welche für die österreichische Lebensmittelindustrie beste Getreidequalitäten geliefert haben. Auch der Export von Qualitätskonsumware hat in Haupt‘s Zeit begonnen stark zu steigen. Wilhelm Haupt hat in seiner aktiven Zeit die positive Entwicklung der Landwirtschaft, besonders in Niederösterreich, mitbeeinflusst.

Wir werden unseren Kollegen und seine Leistungen in dankbarer Erinnerung behalten.

Brandstetter A (2023) Nachruf: Wilhelm Haupt (1934-2022). In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, p 5. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

How plant breeding innovation can help reconciling sustainability with agricultural productivity

Petra JORASCH[1]

[1] Euroseeds, Avenue des Arts 52, 1000 Brussels, Belgium

(✉) petrajorasch@euroseeds.eu

Abstract

Reconciling sustainability with agricultural productivity in the face of climate change relies strongly on the development of resilient, high-yielding crops of superior nutritional value that can be grown more resource efficiently. Therefore, innovation in plant breeding has gained unprecedented importance.

Plant breeding has strongly contributed to increased yields and production in arable farming, and subsequently to improved market and trade conditions, increased food availability, higher economic prosperity and additional farm income while avoiding additional land use, greenhouse gas (GHG) emissions, and loss of biodiversity (Noleppa & Cartsburg, 2021). With this, plant breeding in the European Union drives socio-economic and environmental sustainability. But plant breeding at its current pace will only be able to partially compensate production losses potentially resulting from the implementation of the EU Farm to Fork and Biodiversity strategies. Plant breeding therefor needs to be more efficient to provide farmers with enabling tools (seeds) to sustainably secure their productivity. This requires the availability of a comprehensive breeding toolbox for plant breeders including the latest breeding tools like genome editing to increase efficiency in plant breeding and address breeding goals with the most suitable tools available.

However, the regulatory burden for the latest breeding tools including genome editing is high in Europe. The European Court of Justice (2018) confirmed that organisms obtained by targeted mutagenesis must be considered Genetically Modified Organisms (GMOs) and with this fall under the burdensome and highly politicized EU-GMO approval system under which only one GMO event has ever been approved for cultivation back in 1998.

Applications of the latest breeding tools (new genomic techniques – NGTs) are versatile and can be used in the development of a wide range of different plant products (Jorasch, 2020). While NGTs may for some purposes be used to introduce a transgene and

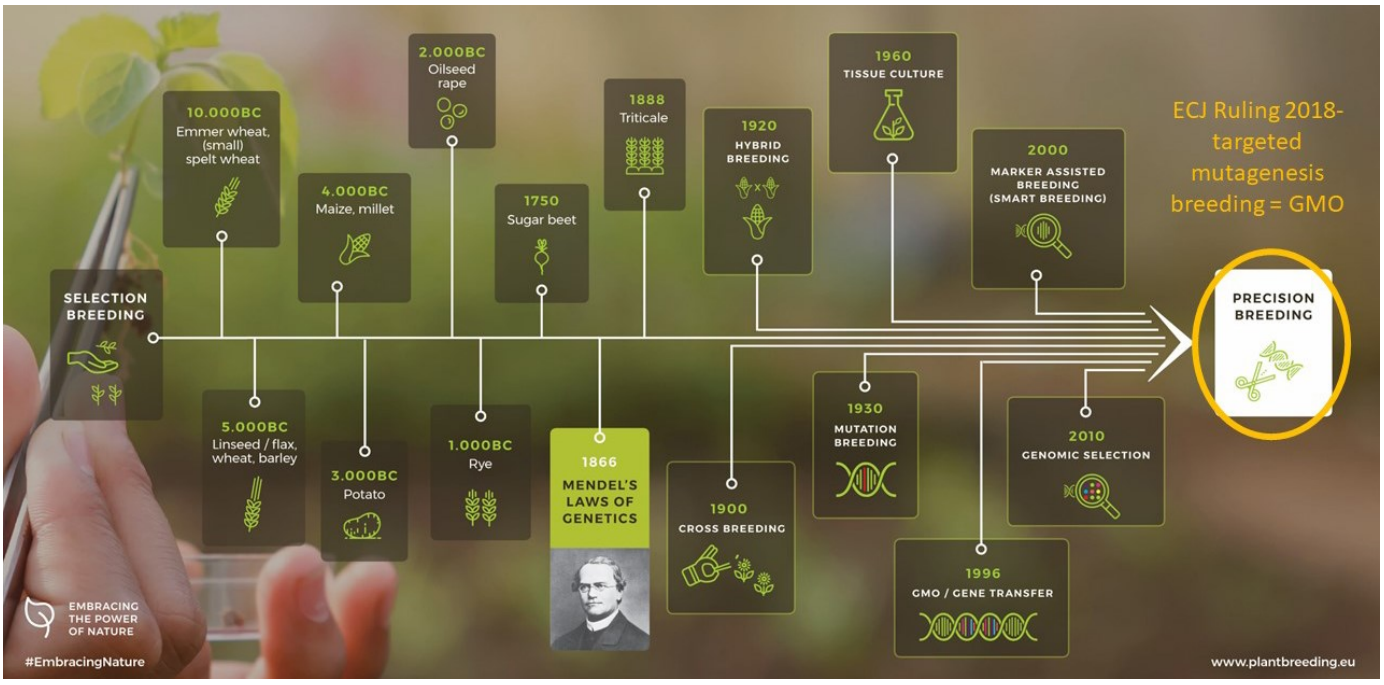

Figure 1 Milestones in plant breeding. Breeders need the full toolbox of efficient and innovative breeding tools available to be able to address breeding goals in the most targeted and efficient way.

Jorasch P (2023) How plant breeding innovation can help reconciling sustainability with agricultural productivity. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 7-8. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

consequently result in a transgenic organism, many other types of NGT-derived plants, *e.g.*, those derived from targeted mutagenesis and cisgenesis, are similar to those that could occur in nature or be produced by conventional breeding methods, *e.g.*, by induced random mutagenesis or backcross breeding (European Commission, 2021). Also, EFSA concluded that certain plants obtained by targeted mutagenesis and cisgenesis do not pose any new hazards compared to plants developed by conventional breeding (EFSA, 2020).

Plant varieties developed through the latest breeding methods should therefore not be subject to different or additional regulations if they could also be obtained through earlier breeding methods or result from spontaneous processes in nature (Euroseeds, 2019). Europe should join the increasing number of countries that follow a differentiated and efficient regulatory approach that allows to clearly determine whether plants resulting from certain NGTs fall into the same category as conventionally bred plants (and thus should be regulated alike) or constitute GMOs according to the respective regulatory framework.

Keywords

Farm to fork · genome editing · GMOs · new genomic techniques · NGTs

References

Court of Justice of the European Union (2018) Judgment of the Court (Grand Chamber), 25 July 2018, in case C-528/16. CJEU, Luxembourg. [https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:62016CJ0528; accessed 24 Jan 2023]

EFSA Panel on Genetically Modified Organisms (2020) Applicability of the EFSA Opinion on site-directed nucleases type 3 for the safety assessment of plants developed using site-directed nucleases type 1 and 2 and oligonucleotide-directed mutagenesis. EFSA J 18:e06299. DOI: 10.2903/j.efsa.2020.6299

European Commission (2021) Study on the status of new genomic techniques under Union law and in light of the Court of Justice ruling in Case C-528/16. EC, Brussels. [https://food.ec.europa.eu/plants/genetically-modified-organisms/new-techniques-biotechnology/ec-study-new-genomic-techniques_en; accessed 24 Jan 2023]

Euroseeds (2019) Position. Plant breeding innovations. Applying the latest plant breeding methods for the benefit of sustainable agriculture, consumers and society. Euroseeds, Brussels. [https://euroseeds.eu/app/uploads/2019/07/18.1010-Euroseeds-PBI-Position-1.pdf; accessed 24 Jan 2023]

Jorasch P (2020) Potential, challenges, and threats for the application of new breeding techniques by the private plant breeding sector in the EU. Front Plant Sci 25:582011. DOI: 10.3389/fpls.2020.582011

Noleppa S, Cartsburg M (2021) The socio-economic and environmental values of plant breeding in the EU and for selected EU member states. An ex-post evaluation and ex-ante assessment considering the “Farm to Fork” and “Biodiversity” strategies. HFFA Research Paper 2021, HFFA Research GmbH, Berlin. [https://hffa-research.com/wp-content/uploads/2021/05/HFFA-Research-The-socio-economic-and-environmental-values-of-plant-breeding-in-the-EU.pdf; accessed 24 Jan 2023]

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Traceability of genome-edited plants and products: Opportunities and challenges

Alexandra RIBARITS[1], Walter STEPANEK[1], Rupert HOCHEGGER[1]

[1] Austrian Agency for Health and Food Safety (AGES), Spargelfeldstr. 191, 1220 Vienna, Austria

(✉) alexandra.ribarits@ages.at

Abstract

The Austrian Agency for Health and Food Safety (AGES) is responsible for implementing European and Austrian legal frameworks with the aim to ensure food safety and food security. Its experts are also active in research and development to provide science-based assessments and to contribute to technological advancements. Controversially discussed topics like genetically modified organisms require specific attention concerning the requirements and expectations of authorities, consumers and the industry. In recent years the discussion on genetically modified organisms has been given a new perspective in view of groundbreaking developments known as "New Breeding Techniques" or "Novel Genomic Techniques" (NGT). Genome editing techniques such as CRISPR/Cas belong to the NGT, which are defined as genetic engineering techniques that have been used since 2001. Plants produced by genome editing are subject to the European framework applying to genetically modified plants (GMP) and products. In this context, AGES provides services like monitoring and surveillance, detection and quantification of impurities, and risk assessment.

Detection and identification of genetically modified organisms is the core requirement for the standard processes in control and surveillance of GMP. In the case of classical GMP, detection typically also includes identification. It is important to differentiate between detection, *i.e*. proving the presence of target sequences by analytical means, and identification, the allocation of detected sequences to specific GMP or derived products. Identification is connected to the uniqueness of such sequences and enables to verify their origin. Screening of typical elements included in GMP, event-specific detection and identification, and quantification of GMP presence are done according to the reference methods published by the European Union Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF). There are some important differences between classical GMP and genome-edited plants that hinder the application of the standard methods. Firstly, screening is not possible as there are no standard screening elements. Secondly, single nucleotide changes can be challenging for reliable detection. Thirdly and most importantly, identification needs detailed consideration. Genome-editing usually does not insert long sequence stretches of foreign or recombinant DNA. As a consequence, no linkage regions between the insert and the plant genome are present in the modified plant. Another major limitation affecting control and surveillance is the unavailability of (certified) reference material.

Basically, the detection of a genome-edited plant is possible, provided that the mutation including its site in the genome is known. On the other hand, its identification may be challenging or even impossible in some cases according to the current state of knowledge and technology. Internationally differing legal requirements may result in marketed genome-edited plants and products. Sufficient and appropriate information about the modification(s) and access to reference material is needed to develop detection methods. It may be necessary to collect additional information to be able to identify a specific genome-edited plant or product. To further enable their traceability, an anticipatory detection and identification framework is proposed. In a knowledge-based approach, different sources of information are fed into a process to support the identification of genome-edited plants or products. Cooperation of competent authorities, governmental agencies, researchers and companies/developers may foster the voluntary disclosure of information and information exchange. EUginius (EUropean GMO INItiative for a Unified Database System) is a European initiative with the intention to support the competent authorities and private users. The EUginius database provides accurate information about genetically modified organisms (GMO) and contains a non-exhaustive list of organisms developed with NGT. Currently, the database shows forty-six entries of genome-edited plants, including several important crop species (Fig. 1). An international database to collect the relevant information and making use of best practice examples (governance of food products, stewardship programs, seed certification, etc.) may be a good basis for a detection and identification network that ensures the traceability of genome-edited plants.

Keywords

Database · detection · feed · food · identification · seed

Ribarits A, Stepanek W, Hochegger R F (2023) Traceability of genome-edited plants and products: Opportunities and challenges. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 9-10. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

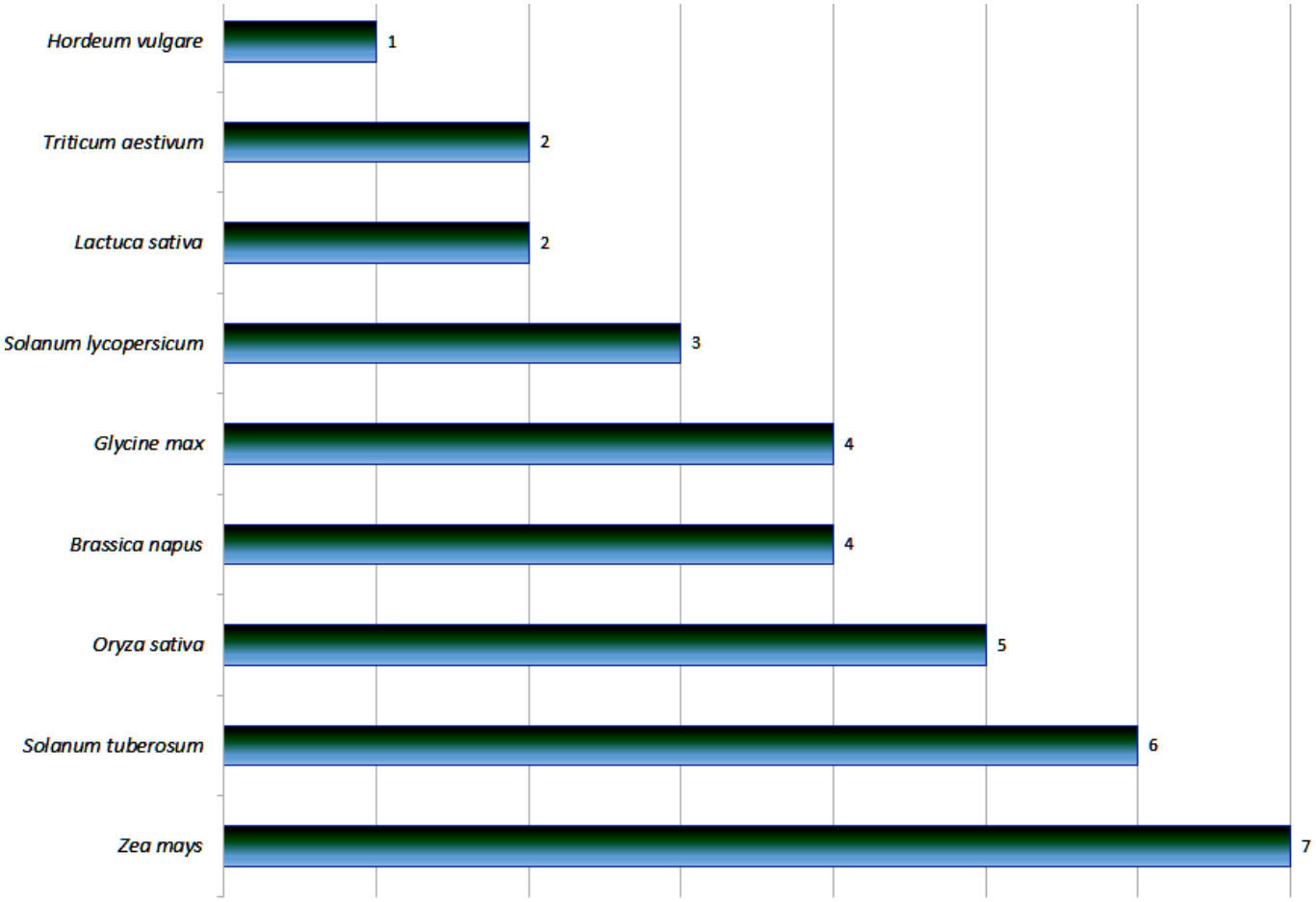

Figure 1 Selected important crop species and their respective number of entries in the EUginius database, as of January 2023.

Acknowledgements

This project was commissioned by the German Federal Agency for Nature Conservation (BfN) with funds from the German Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) (Research and Development Grant No. (FKZ) 3519801000, "Nachweismethoden für genomeditierte und klassische GV-Pflanzen").

References and further reading

EUginius (2023) The European GMO database. Federal Office of Consumer Protection and Food Safety, Braunschweig & Wageningen Food Safety Research, Wageningen University & Research. [https://euginius.eu; accessed 27 January 2023]

Ribarits A, Stepanek W, Hochegger R, Narendja F, Prat N, *et al*. (2022) Analyse von Nachweismethoden für genomeditierte und klassische GV-Pflanzen. BfN-Skripten 622. Bundesamt für Naturschutz, Bonn. [https://www.bfn.de/sites/default/files/2022-02/Skript622.pdf; accessed 12 January 2023]

Ribarits A, Eckerstorfer M, Simon S, Stepanek W (2021) Genome-edited plants: Opportunities and challenges for an anticipatory detection and identification framework. Foods 10:430. DOI: 10.3390/foods10020430

Ribarits A, Narendja F, Stepanek W, Hochegger R (2021) Detection methods fit-for-purpose in enforcement control of genetically modified plants produced with novel genomic techniques (NGTs). Agronomy 11:61. DOI: 10.3390/agronomy11010061

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

CRISPR/Cas9 mediated genome editing in barley and wheat

Eszter KAPUSI[1], Barbara STEINER[2], Hermann BÜRSTMAYR[2], Eva STÖGER[1]

[1] University of Natural Resources and Life Sciences, Vienna, Department of Applied Genetics and Cell Biology, Muthgasse 11, 1190 Vienna, Austria

[2] University of Natural Resources and Life Sciences, Vienna, Department of Agrobiotechnology, IFA-Tulln, Konrad Lorenz Str. 20, 3430 Tulln an der Donau, Austria

(✉) eszter.kapusi@boku.ac.at

Abstract

In the genotype screening of primary regenerants of barley (*Hordeum vulgare*), PCR was carried out to select plants positive for Cas9. Then primary transformants containing the cas9 gene were analyzed to determine whether there were any Cas9-triggered double-strand breaks (DSBs) in the putative barley ENGase gene. The T1 progeny were similarly analyzed to determine whether the induced mutations were heritable. After sequencing of selected single target amplicons (integrated in an intermediate vector) the mutations can be exactly characterized. As expected, the typical outcome of CRISPR/Cas9 editing in the primary regenerants was the insertion or deletion of several bases either at one target site, in some at both of a pair. In several plants, both target sites were affected by the active Cas9 nuclease resulting in larger chromosomal fragment deletions, which could be detected without sequencing. These mutations can be homozygous, heterozygous, or biallelic. Plants were identified with up to six different induced mutations, which reveals chimeric state of the T0 regenerant. This means that these regenerants consist of different transgenic tissues carrying different induced DSBs. Ample T1 lines were identified where the transgene (cas9:hpt) segregated independently from the induced indels and fragment deletions. This resulted in the production of trangsene-free progeny carrying the desired mutations without concerns for consecutive mutations induced by an active Cas9. An edited trait can be fixed by using doubled haploids (DH). Immature pollen culture is able for the generation of instantly homozygous breeding lines for the transmitted editing. The consecutive step is to test the consequence of the lacking expression of the ENGase in the glycosylation pattern of a recombinant protein. For this, lines expressing the heavy and light chains of the 2G12 antibody in barley were crossed with ENGase knock-out lines. The hybrid progeny contained the 2G12 gene and an ENGase allele with fragment deletion in hemizygous/heterozygous state. In order to produce instantly homozygous lines for the two genes-of-interest, the DH technique was applied. Regenerants expressing the 2G12 antibody and induced mutations in the ENGase target area were selected. These plants were analyzed for their 2G12 glycosylation pattern and to evaluate, if the polypeptide chain of the antibody indeed lacks GlcNAc residues. However, the functionality of the putative barley ENGase could not be verified until now.

Wheat (*Triticum aestivum*) is considered to be the world's most important crop, is one of the "big three" cereal crops together with maize (*Zea mays*) and rice (*Oryza sat*iva). Genetic engineering is expected to play a crucial role in its further improvement. However, wheat is also the most difficult to genetically transform or edit out of the main cereal crops due to its large genome size and hexaploid state. The recalcitrance of many elite varieties is making wheat genome editing challenging.

Fusarium head blight (FHB) is a fungal disease that leads to severe losses in both yield and quality of cereals. Moreover, the fungus produces mycotoxins that accumulate in the grains. Resistance of wheat to FHB is a quantitative trait with only a few causal genes isolated until now. A donor line resistant to FHB spreading (type II resistance) and carrying a specific genomic region compared to the reference 'Chinese Spring' was selected for genome editing. CRISPR/Cas-induced mutagenesis of selected candidate genes in this region will verify their role in the fungal disease, and to analyze their effects on FHB spreading and mycotoxin resistance. Despite poor genetic transformation efficiencies, primary regenerants containing the CRISPR/Cas9 system components were identified, and one plant revealed a 5 bp deletion at the site targeted by a sgRNA. The induced mutation was verified by a restriction digestion assay, in which the BanI enzyme recognition sequence was disrupted by the editing and remained unaffected upon enzyme addition. Experiments are currently underway to generate additional mutants and to functionally evaluate the candidate genes.

Keywords

ENGase · Fusarium head blight · genome editing · *Hordeum vulgare* · induced mutation · protein glycosylation · *Triticum aestivum*

Kapusi E, Steiner B, Bürstmayr H, Stöger E (2023) CRISPR/Cas9 mediated genome editing in barley and wheat. In: Vereinigung der Pflanzenzüchter und Saatgut-kaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 11-12. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

Acknowledgements

The authors would like to acknowledge financial support by the Austrian Science Fund FWF (P 34353).

References and further reading

Buerstmayr M, Steiner B, Buerstmayr H (2020) Breeding for Fusarium head blight resistance in wheat - Progress and challenges. Plant Breed 139:429-454. DOI: 10.1111/pbr.12797

Kapusi E, Corcuera-Gómez M, Melnik S, Stoger E (2017) Heritable genomic fragment deletions and small indels in the putative ENGase gene induced by CRISPR/Cas9 in barley. Front Plant Sci 8:540. DOI: 10.3389/fpls.2017.00540

Matres JM, Hilscher J, Datta A, Armario-Nájera V, Baysal C, *et al*. (2021) Genome editing in cereal crops: an overview. Transgenic Res 30:461-498. DOI: 10.1007/s11248-021-00259-6

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Development of plant breeding innovations based on natural diversity – Mission impossible?

Bernd HACKAUF[1]

[1] Julius Kühn-Institute, Federal Research Centre for Cultivated Plants, Institute for Breeding Research on Agricultural Crops, Rudolf-Schick-Platz 3a, 18190 Groß Lüsewitz, Germany

(✉) bernd.hackauf@julius-kuehn.de

Abstract

The discovery of Mendel's laws of heredity in the 19th century transformed the ancient activity of selective methods from early plant-breeding procedures, dating to the very beginnings of agriculture, to a science. One of the major basic essentials that emerged during the history of scientific plant breeding is the knowledge about the tremendous wealth of genetic variability existing in plants. With an increased understanding of plant biology and plant genes, plant breeders have continuously improved their breeding tools for tapping the potential of genetic diversity to adapt plants to the ever-changing demands of farmers, consumers, and the environment. New Genomic Techniques (NGTs) are tools in plant breeding that make use of genetic diversity by induced mutagenesis directed to a defined genomic location, thus enabling editing of the genome with a precision not feasible before. NGTs render it possible to create genome alterations directly in elite germplasm and promise to shorten the development time for improved cultivars with desired phenotypes. Knowledge about causal sequence motifs for phenotypic variation is the indispensable prerequisite for changing the genetic material of a plant in the desired direction.

The availability of high-quality reference genome sequences offers a state-of-the-art framework for identifying functional sequence motifs even in neglected crops like rye (Rabanus-Wallace *et al*. 2021). Indeed, a genome-wide association study (GWAS) has mapped 676 cross-validated SNPs associated with complex agronomic and quality traits to the Lo7 pseudomolecules with 206 SNPs residing in coding sequences (Siekmann *et al*. 2021). Follow-up studies are necessary to advance these GWAS results and to infer the exact causal variants. However, going from genetics to function is a challenging task in rye. For reverse-genetic tools to be functional, its active components have to be delivered to a cell for creating genetic modifications and observing the resulting phenotype. In rye, efficient procedures to investigate the impact of induced variation within a specific gene and to infer gene function by gene silencing, homologous recombination or non-targeted random disruptions (*e.g*. chemical mutagenesis or transposon mediated mutagenesis) followed by screening a library of individuals for mutations at a specific location are lacking. In a case study unlocking a major QTL controlling fertility restoration of Pampa-CMS in rye, methods to identify the candidate gene such as fine mapping of the mendelian factor underlying the QTL (Hackauf *et al*. 2012) as well as high-resolution genetic and physical mapping, and allele sequencing (Wilde *et al*. 2021) have been successfully applied. However, conclusive evidence for male-fertility restoration of genetic variants in the identified candidate gene by specific functional assays is owing, as delivery mechanisms for gene-editing that bypass current challenges in tissue culture and regeneration procedures of rye need to be established.

As plant-breeding innovations like NGTs seem to be not applicable at least in the short term, improved breeding efforts are crucial for enhancing the competitiveness of rye in agricultural production systems. The natural genetic diversity in rye was the fundamental basis to achieve a series of technological advances that ultimately facilitated the establishment of hybrid breeding, a cutting-edge technology to increase and secure cereal production on finite arable land without increasing water and fertilizer use (Hackauf *et al*. 2022). The recent implementation of the Gibberellin-sensitive dominant dwarfing gene *Ddw1* in a commercial hybrid rye breeding program is paradigmatic for specific genes, and adaptive alleles that govern important agronomic traits in rye. The promising performance observed in extreme environments in 2021 and 2022 triggers an enhanced development of semidwarf hybrids and may initiate a new era of physiological rye breeding that aims to raise the yield potential of rye closer to its biological limit (Hackauf *et al*. 2022). To conclude, the successful integration of a Mendelian inheritance factor in hybrid rye breeding improves yield potential, lodging resistance, and drought tolerance and is an up-to-date example of reconciling food security and sustainability through plant breeding innovations based on natural diversity.

Keywords

Agronomic traits · GWAS · hybrid breeding · Mendelian inheritance · reference genome sequence · *Secale cereale*

Hackauf B (2023) Development of plant breeding innovations based on natural diversity - Mission impossible? In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 13-14. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

Acknowledgements

The development of the semidwarf seed-parent lines was funded by the German Federal Ministry of Food and Agriculture based on the decision of the Parliament of the Federal Republic of Germany through the Federal Office of Agriculture and Food (Grant No. 2814IP001). The project RYE-SUS has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No. 771134 for the development and evaluation of semidwarf rye hybrids. RYE-SUS was carried out under the ERA-NET Cofund SusCrop (Grant No. 771134), being part of the Joint Programming Initiative on Agriculture, Food Security and Climate Change (FACCE-JPI).

References and further reading

Hackauf B, Korzun V, Wortmann H, Wilde P, Wehling P (2012) Development of conserved ortholog set markers linked to the restorer gene *Rfp1* in rye. Mol Breed 30:1507-1518. DOI: 10.1007/s11032-012-9736-5

Hackauf B, Siekmann D, Fromme FJ (2022) Improving yield and yield stability in winter rye by hybrid breeding. Plants 11:2666. DOI: 10.3390/plants11192666

Rabanus-Wallace MT, Hackauf B, Mascher M, Lux T, Wicker T, *et al*. (2021) Chromosome-scale genome assembly provides insights into rye biology, evolution, and agronomic potential. Nat Genet 53:564-573. DOI: 10.1038/s41588-021-00807-0

Siekmann D, Jansen G, Zaar A, Kilian A, Fromme FJ, *et al*. (2021) A genome-wide association study pinpoints quantitative trait genes for plant height, heading date, grain quality, and yield in rye (*Secale cereale* L.). Front Plant Sci 12:718081. DOI: 10.3389/fpls.2021.71808

Wilde P, Korzun V, Menzel J, Zhou R, Stein N, *et al*. (2021) Restorer-Pflanze, Restorer-Plants, Plante Restauratrice. KWS SAAT SE, EP 2016/082268, 21 Dec 2016. Europäisches Patentblatt 50/2021: 790.

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Input of new breeding technologies to the dissection of plant traits genetics and the development of new varieties: 5-years literature analysis

Lyudmyla MALYSHEVA-OTTO[1]

[1] CEO, IT-Breeding GmbH, BGI Biotechpark Gatersleben, Am Schwabeplan 6, OT Gatersleben, 06466 Seeland, Germany

(✉) malysheva-otto@it-breeding.de

Abstract

To examine the input of new breeding techniques (NBTs) in practical plant breeding, an analysis of literature was performed for the period of 5 years, *i.e*. 2017 to 2021, with the focus on two important crops, *i.e*. maize (*Zea mays*) and rapeseed (*Brassica napus*). Alongside marker-assisted breeding methods that are meanwhile generally adopted and wide spread, *e.g*. marker-assisted selection (MAS), marker-assisted back-crossing (MABC), genomic selection (GS) and genomic prediction (GP), etc., also new genomic techniques (NGTs), *i.e*. genome editing, were considered.

Among, in total, almost 7000 scientific articles, about 3000 for rapeseed and 4000 for maize, published during the last 5 years, about 130 and 190, respectively, utilized NBTs and were relevant for the present study. Table 1 gives an overview of the analysis.

To conclude, the application of new breeding and genomic techniques allowed the identification of hundreds of candidate genes for important agronomic traits. Some genes were verified and confirmed in diverse plant panels and used for the development of highly associated molecular markers, which can be directly applied in marker-assisted breeding. Genome editing technologies proved to be very effective for the functional gene annotation as well as as for the manipulation of the plant phenotype, although changes of the phenotype was hitherto achieved for a limited number of traits.

Further studies will pave the way for optimized and publicly accepted strategies for variety development, allowing the accelerated development of new varieties with improved traits necessary for securing food supplies in face of climate change.

Table 1 Overview of genomics research supporting plant breeding

Rapeseed	Maize
Strategies implemented to unravel genetic background of traits and to breed new varieties (Number of publications):	
QTL mapping (54)	QTL mapping (68)
GWAS (38)	GWAS (76)
Candidate genes/Regional association study	Candidate genes/Regional association study
QTL-seq	QTL-seq
Genomic Selection	Genomic Selection
Associative transcriptomics	Single plant GWAS (sp-GWAS)
EMS mutagenesis	
Integration of different strategies (16):	*Integration of different strategies (22):*
Combined genetic analysis, gene mapping, sequence analysis	Combined genetic analysis, map-based cloning
Combined QTL mapping, whole genome sequencing, transcriptome analysis (gene expression, RNA-Seq), eQTL	Combined QTL mapping, GWAS, QTL-Seq, transcriptome analysis, candidate regional association mapping,
Combined GWAS, transcriptome sequencing, gene co-expression network analysis	Combined GWAS, transcriptome sequencing, eQTL mapping, pathway-level analysis, map-based cloning
Combined bulk segregant analysis, whole-genome resequencing, SLAF-Seq, transcriptome analysis, map-based cloning	Combined bulk segregant sequencing, QTL mapping

Malysheva-Otta L (2023) Input of new breeding technologies to the dissection of plant traits genetics and the development of new varieties: 5-years literature analysis. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 15-16. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

Table 1 Continued

Rapeseed	Maize
Application of the CRISPR/Cas9-based technologies:	
For functional gene analysis (6)	For functional gene analysis (12)
To achieve plants with improved phenotype (7)	To achieve plants with improved phenotype (6)
Candidate genes/Regional association study	Candidate genes/Regional association study
Genotyping methods applied:	
SNP arrays	SNP arrays
Genotyping-by-sequencing (GBS)	Genotyping-by-sequencing (GBS)
SSR genotyping	SSR genotyping
SLAF-seq (specific-locus amplified fragment sequencing)	RNA-seq Genotyping by target sequencing (GBTS), available as targeted sequence-based genotyping solution from KeyGene, SNPSelect
Outcome for direct/indirect utilization in breeding programmes:	
Candidate genes identified or suggested (55)	Candidate genes identified or suggested (130); sequence analysis of the candidate gene in diversity panel
Markers developed (10)	Markers developed (8); MABC and MAS; marker-assisted stacking/pyramiding
Improved phenotypes achieved by CRISPR/Cas9 (7)	Improved phenotypes achieved by CRISPR/Cas9 (6)
Target phenotypes:	
Plant architecture and increased yield, early flowering, fatty acid profile, multilocular phenotype	Male sterility, waxy starch, targeted crossover induction

Keywords

Brassica napus · genome editing · marker-assisted breeding · *Zea mays*

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

WheatSustain – an example of a fruitful transnational collaboration between academia and industry to develop genomics-based breeding tools for practical application in wheat breeding

Morten LILLEMO[1], Hermann BUERSTMAYR[2], Lorenz HARTL[3], Julio ISIDRO-SÁNCHEZ[4,§], Maria Antonia HENRIQUEZ[5], Josef HOLZAPFEL[6], Anja HANEMANN[7], Deniz AKDEMIR[8], Muath ALSHEIKH[9], Jose CROSSA[10], Franziska LÖSCHENBERGER[11]

[1] Norwegian University of Life Sciences, Faculty of Biosciences, 1433 Ås, Norway
[2] University of Natural Resources and Life Sciences, Vienna, Dept. of Agrobiotechnology, IFA-Tulln, Konrad Lorenz Str. 20, 3430 Tulln, Austria
[3] Bavarian State Research Center for Agriculture, Institute for Crop Science and Plant Breeding, Am Gereuth 8, 85354 Freising, Germany
[4] University College Dublin, Animal and Crop Science, Belfield, Dublin 4, Ireland
[5] Agriculture and Agri-Food Canada, Morden Research and Development Centre, 101 Route 100, R6M 1Y5 Morden, Manitoba, Canada
[6] Secobra Saatzucht GmbH, Feldkirchen 3, 85368 Moosburg, Germany
[7] Saatzucht Josef Breun GmbH & Co. KG, Amselweg 1, 91074 Herzogenaurach, Germany
[8] StatGen Consulting, 1315 Coddington Rd., 14817 Brooktondale, NY, USA
[9] Graminor AS, Hommelstadvegen 60, 2322 Ridabu, Norway
[10] International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600 Mexico City, Mexico
[11] Saatzucht Donau GesmbH & CoKG, Saatzuchtstr. 11, 2301 Probstdorf, Austria
[§] Current address: Universidad Politécnica de Madrid (UPM), Centro de Biotecnología y Genómica de Plantas, 28223 Madrid, Spain

(✉) morten.lillemo@nmbu.no

Abstract

Genomic selection allows for a faster and more effective breeding of new cultivars by utilizing high-density marker data to predict breeding values of progeny lines. Much room exists to improve the genomic prediction of complex disease resistances such as stripe rust (*Puccinia striiformis* f.sp. *tritici*) and Fusarium Head Blight (FHB) in wheat. The core idea behind the SusCrop ERA-NET project *WheatSustain* (2019-2022) was to make use of biologically relevant data, known trait correlations, environmental effects and quantitative trait loci (QTL) to build more robust genomic prediction models for use in genomic selection. In the long run, this will enable breeders to develop new cultivars with improved disease resistance, which will suffer less yield and quality losses and can be cultivated with less use of fungicides.

WheatSustain established a close collaboration among world leading experts on genomic prediction modeling, bioinformatics, wheat genomics and leaders in the field of plant pathology and genetics of stripe rust and FHB resistance in wheat. Research groups from Norway, Ireland, Germany, Austria, Mexico, USA and Canada have worked in close collaboration with public and private wheat breeding programmes. Plant breeders have taken active part in the research by providing germplasm, conducted field trials, and validated molecular markers and genomic prediction models in their breeding programmes.

To facilitate seamless exchange of data among the partners, a common database was established. We also merged project data with previously existing data and public repositories, using a new data harmonization approach that we published. We also developed methodology for the optimal design of training populations, which is a crucial question for the application of genomic selection in plant breeding. The R-package TrainSel was developed for this purpose and published open source. Computer simulations were done to compare alternative approaches to applying genomic selection for disease resistance. Common winter wheat and spring wheat training populations were tested for FHB and stripe rust resistance in field trials across the partner countries using standardized field scoring protocols. Moreover, seedling assays were conducted for stripe rust. Genome-wide association studies (GWAS) yielded new knowledge and insight into the genetics of the resistance to these two important diseases in the most relevant winter wheat and spring wheat germplasm. Markers for the most important QTL as well as genomic prediction models were further validated on new breeding lines and are now being used as selection tools by the collaborating breeding companies.

The work on stripe rust revealed that seedling resistance correlated poorly with adult plant resistance in the field. Although seedling data from inoculations with single isolates are important

Lillemo M, Buerstmayr H, Hartl L, Isidro-Sánchez J, Henriquez MA, *et al*. (2023) WheatSustain – an example of a fruitful transnational collaboration between academia and industry to develop genomics-based breeding tools for practical application in wheat breeding. In: Vereinigung der Pflanzenzüchter und Saatgut-kaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 17-18. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

for distinguishing race specific and race non-specific resistance, genomic prediction models need to be trained on field data to be reliable as selection tools in breeding.

For FHB, our modeling concepts of using correlated traits as co-variates in the prediction models were confirmed on both the winter wheat and spring wheat data. The take-home message for breeders is to make use of plant height, anthesis date and anther extrusion in multi-trait genomic prediction models to achieve the best prediction accuracy.

In summary, the project has fostered a very fruitful transnational collaboration between academic research institutes and breeding companies. Outputs at the end of the project in 2022 included a total of 14 research papers in international journals and 14 conference presentations, as well as several manuscripts under preparations for publication. More importantly, the project has led to validated molecular markers for disease resistance and genomic prediction concepts that are being applied in variety development by the collaborating breeding companies. The concepts developed in *WheatSustain* can easily be transferred and adapted to other disease resistance traits and crop species.

Keywords

Fusarium head blight (FHB) · genomic prediction · genomic selection · stripe rust · *Triticum aestivum*

Acknowledgements

The WheatSustain project was carried out under the ERA-NET Cofund SusCrop (Horizon 2020 Grant No 771134), being part of the Joint Programming Initiative on Agriculture, Food Security and Climate Change (FACCE-JPI). Funding for the project partners came from the Research Council of Norway (NFR grant 299615), Deutsches Bundesministerium für Bildung und Forschung (031B0810), Bundesministerium für Nachhaltigkeit und Tourismus Österreich (Forschungsprojekt Nr. 101402), the Genome Canada project CTAG2, and the Canadian Agricultural Partnership administered by the Canadian Wheat Research Coalition, and the Department of Agriculture, Food and the Marine (DAFM), Ireland.

References and further reading

Akdemir D, Knox R, Isidro y Sánchez J (2020) Combining partially overlapping multi-omics data in databases using relationship matrices. Front Plant Sci 11:947. DOI: 10.3389/fpls.2020.00947

Akdemir D, Rio S, Isidro y Sánchez J (2021) TrainSel: An R package for selection of training populations. Front Genet 12:655287. DOI: 10.3389/fgene.2021.655287

Garcia-Abadillo J, Morales L, Buerstmayr H, Michel S, Lillemo M, *et al*. (2023) Alternative scoring methods of fusarium head blight resistance for genomic assisted breeding. Front Plant Sci 13:1057914. DOI: 10.3389/fpls.2022.1057914

Nannuru VKR, Windju SS, Belova T, Dieseth JA, Alsheikh M, *et al*. (2022) Genetic architecture of fusarium head blight disease resistance and associated traits in Nordic spring wheat. Theor Appl Genet 135:2247-2263. 10.1007/s00122-022-04109-9

Shahinnia F, Geyer M, Schürmann F, Rudolphi S, Holzapfel J, *et al*. (2022) Genome-wide association study and genomic prediction of resistance to stripe rust in current Central and Northern European winter wheat germplasm. Theor Appl Genet 135:3583–3595. 10.1007/s00122-022-04202-z

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Assessing single and multi-trait genomic prediction model accuracies including significant GWAS markers for Fusarium head blight disease resistance in wheat (*Triticum aestivum*)

Vinay Kumar Reddy NANNURU[1], Jon Arne DIESETH[2], Curt A. McCARTNEY[3], Maria Antonia HENRIQUEZ[3], Hermann BUERSTMAYR[4], Sebastian MICHEL[4], Laura MORALES[4], Theo H. E. MEUWISSEN[5], Jose CROSSA[6], Morten LILLEMO[1]

[1] Norwegian University of Life Sciences, Faculty of Biosciences, 1433 Ås, Norway

[2] Graminor AS, Hommelstadvegen 60, 2322 Ridabu, Norway

[3] Agriculture and Agri-Food Canada, Morden Research and Development Centre, 101 Route 100, R6M 1Y5 Morden, Manitoba, Canada

[4] University of Natural Resources and Life Sciences, Vienna, Dept. of Agrobiotechnology, IFA-Tulln, Konrad Lorenz Str. 20, 3430 Tulln, Austria

[5] Norwegian University of Life Sciences, Department of Animal and Aquacultural Sciences, 1433 Ås, Norway

[6] International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600 Mexico City, Mexico

(✉) morten.lillemo@nmbu.no

Abstract

Fusarium head blight (FHB) is a widely known devasting disease of wheat caused by *Fusarium graminearum* and other *Fusarium* species. FHB can cause severe yield losses due to failed kernel development or because infected kernels are shrivelled, discoloured and low in test weight. *F. graminearum* which produces the mycotoxin deoxynivalenol (DON) is found to be the most causal agent of FHB in wheat. Mycotoxins, such as DON 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 mechanisms is required. FHB resistance is quantitative, highly complex and divided into several resistance types. Resistance to FHB has been divided into active and passive resistance mechanisms. 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. A genome-wide association study (GWAS) by Nannuru *et al*. (2022) conducted on a NMBU spring wheat panel detected thirteen robust QTL regions that could be important for FHB resistance. Previously, several studies used GWAS associated markers in genomic prediction models to improve the predictive ability of the prediction models and were successful. We have used a similar approach in this study to improve the predictive ability of the genomic selection models. Genomic prediction (selection; GS) is a method used to predict the genetic value/ breeding value of genotypes (without phenotypes) based on the estimated association between phenotype and genome-wide markers. The predicted breeding value is normally called Genomic Estimated Breeding Values (GEBVs) and selection is done using the GEBVs.

Data was obtained from field trials on two wheat panels: the NMBU spring wheat panel (hereafter referred to as NMBU panel), and the Graminor spring wheat panel (hereafter referred to as Graminor panel). The NMBU panel is a collection of 296 hexaploid spring wheat accessions including lines mainly from Norway, Europe, USA, CIMMYT (Mexico), China and Australia. The Graminor panel consists of 358 new breeding lines from the commercial spring wheat breeding program of Graminor. The NMBU panel was tested over five years in four different locations, whereas the GRAMINOR panel was tested over two years in three locations.

The objective of this study was to train different models and predict the breeding values for ST and MT models by incorporating the GWAS based significant SNP markers as a fixed effect into the genomic prediction models. Genomic prediction models used are as follows: (i) base GS model, without GWAS markers ($y = \mu + Zu + e$) and (ii) base + GWAS GS model with GWAS markers ($y = \mu + X\beta + Zu + e$). Single-trait and multi-trait genomic prediction models (ST & MT). Cross-validation was done using five-fold in ten replications. Prediction accuracy was determined as the Pearson correlation between GEBVs and observed phenotypes.

Results showed enhanced prediction ability of the GS models with GWAS associated markers. Especially the prediction accuracies were higher for ST + GWAS when compared to MT + GWAS. When correlated multiple traits were included, the prediction accuracies remained unaffected in most of the cases. But the prediction accuracies for MT GS models were comparatively higher than ST, whether GWAS markers were included or not. This substantial increase in prediction ability of MT models was due to the borrowed phenotypic information from the correlated traits (Fig. 1).

Nannuru VKR, Dieseth JA, McCartney CA, Henriques MA, Buerstmayr H, *et al*. (2023) Assessing single and multi-trait genomic prediction model accuracies including significant GWAS markers for Fusarium head blight disease resistance in wheat (*Triticum aestivum*). In: Vereinigung der Pflanzenzüchter und Saatgut-kaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 19-20. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

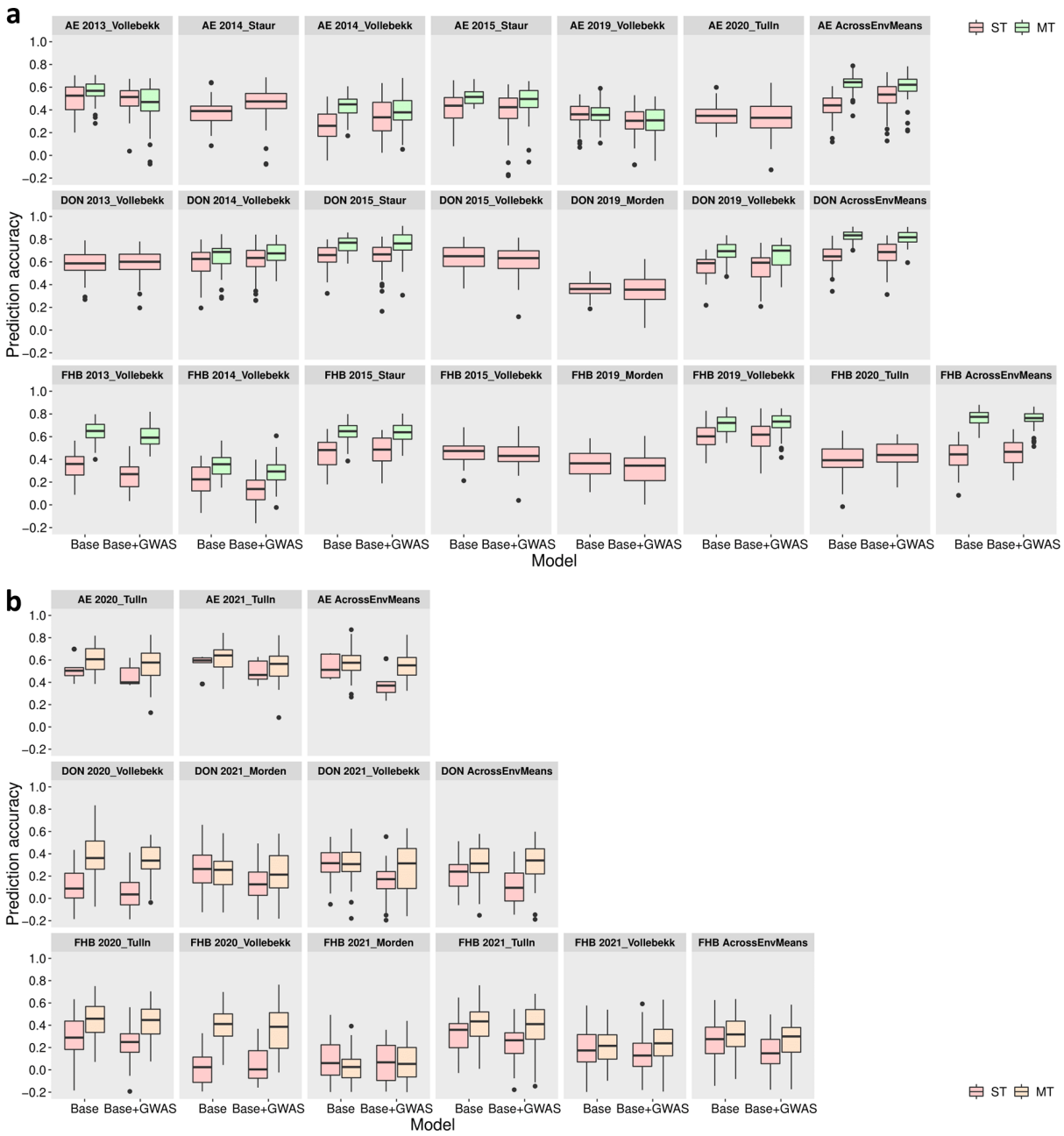

Figure 1 Boxplots showing prediction accuracies for single environments (Year×Location) and across-environment means (AcrossEnvMeans) of anther extrusion (AE; top row), deoxynivalenol content (DON; center row) and Fusarium head blight severity (FHB; bottom row) using single trait and multi-trait prediction models in **a** the NMBU panel and **b** the Graminor panel. X-axis labelling: Base = standard conventional genomic selection (GS) model; Base+GWAS = GS model including GWAS associated markers as fixed covariates; Boxes: ST = single trait; MT = multi trait.

This study concludes that the utility of incorporating GWAS results in genomic prediction modelling is noteworthy for complex disease traits when GWAS findings have captured useful information from the genomic regions in the form of quantitative trait nucleotids (QTNs). Consequently, it also concludes multi-trait genomic prediction models are advantageous over single-trait models when the associated correlated traits are used in predicting complex disease resistance traits .

Keywords

Fusarium head blight (FHB) · genomic prediction · genomic selection · GWAS SNP covariate

References and further reading

Nannuru VKR, Windju SS, Belova T, Dieseth JA, Alsheikh M, *et al*. (2022) Genetic architecture of Fusarium head blight disease resistance and associated traits in Nordic spring wheat. Theor Appl Genet 135: 2247-2263. DOI: 10.1007/s00122-022-04109-9

Sehgal D, Rosyara U, Mondal S, Singh R, Poland J, *et al.* (2020) Incorporating genome-wide association mapping results into genomic prediction models for grain yield and yield stability in CIMMYT spring bread wheat. Front Plant Sci 11:197. DOI: 10.3389/fpls.2020.00197

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Evaluating genomic selection and speed breeding for Fusarium head blight resistance in wheat using stochastic simulations

Vinay Kumar Reddy NANNURU[1], Jon Arne DIESETH[2], Morten LILLEMO[1], Theo H. E. MEUWISSEN[3]

[1] Norwegian University of Life Sciences, Faculty of Biosciences, 1433 Ås, Norway

[2] Graminor AS, Hommelstadvegen 60, 2322 Ridabu, Norway

[3] Norwegian University of Life Sciences, Department of Animal and Aquacultural Sciences, 1433 Ås, Norway

(✉) theo.meuwissen@nmbu.no

Abstract

Acceleration of the breeding cycle with higher genetic gains and reduced operating costs is possible by the application of novel approaches into the breeding programme. The availability of high-density low-cost marker genotyping platforms makes genomic prediction, and selection feasible. Genomic selection (GS) (Meuwissen *et al*. 2001) can be implemented in the breeding programme in order to predict 'breeding values' of progeny lines without costly phenotyping, saving time and money, as well as increasing selection intensity and accuracy of trait prediction. Stochastic simulations which are used to evaluate breeding programmes virtually are gaining attention in plant breeding. These simulations can be used to develop and simulate entire breeding programmes artificially mimicking the real breeding programme with user-defined parameters. Such simulations can help breeders to predict the outcome of alternative breeding schemes. Integrating GS in plant breeding programmes and testing their potential can be assessed with the help of these simulations prior to its real implementation which could be a big investment in terms of labour and time with no satisfactory results. GS can be integrated with speed breeding which can further help to reduce the length of breeding cycle. This idea was already investigated by Voss-Fels *et al*. (2019) using such simulations.

The genome sequence was simulated using real genotypic data (Genetic map) from a set of wheat breeding lines called (MASBASIS) developed for more than a decade at the Norwegian breeding company Graminor. The founder population has a size of 100 individuals. A 40 year breeding programme was simulated including 20 years of a 'burn-in phase' with traditional phenotypic selection and another 20 years of 'breeding advancement phase' consisting of four alternative schemes: (i) phenotypic selection, (ii) GS-F_7, (iii) GS-F_2 & F_7, and (iv) speed breeding + GS. Parents are updated in the crossing block in the next breeding cycle. Twenty old parents from previous cycle's crossing block are updated with the best performing candidate lines from the advanced yield trial stage. Performance is based on the respective breeding values in case of the phenotypic selection breeding scheme and by using estimated breeding values in case of GS related schemes. Two different approaches were used for the selection of candidate lines based on performance measured in terms of breeding values or estimated breeding values. In the 'single trait approach' candidate lines were selected only based on a single trait of importance, in the 'selection index approach' correlated traits are used for selecting the candidate lines based on weights and the Smith-Hazel index.

Objectives of this simulation study were (i) to examine the potential benefits of GS over traditional phenotypic selection in terms of breeding gains and (ii) comparing the combination of speed breeding with GS to other breeding schemes.

Different breeding schemes were evaluated for change of genetic gain and change of genetic variance over the time. Genetic gain was measured as the mean genetic value of total individuals in F_8 for trait deoxynivalenol (DON) content. Change of genetic gain over the time of 20 years was evaluated, where speed breeding + GS showed higher genetic gain change over time. Genetic gain was further enhanced using the selection index approach (Fig. 1). Genetic variance was measured as the mean genetic variance of total individuals in F_8 for DON content. Change of genetic variance over the time of 20 years was evaluated, where speed breeding + GS had greater change in genetic variance over the time. Genetic variance change did not vary much among the schemes using selection index approach (Fig. 1).

GS is advantageous over phenotypic selection in terms of achieving breeding goals faster using stochastic simulation. Combining GS with speed breeding further enhances breeding gains. There is a potential benefit to implement the schemes evaluated in these simulations in a practical breeding programme with the wide availability of cheap genotyping platforms and speed breeding protocols. The simulations also showed that multi-trait selection using a selection-index with pre-defined weights based on the economic importance of the traits is preferable over single-trait selection. We strongly believe that adopting GS in plant breeding programmes would be beneficial with a small compromise on initial costs, that can return good profits with widespread and successful research development of GS.

Nannuru VKR, Dieseth JA, Lillemo M, Meuwissen THE (2023) Evaluating genomic selection and speed breeding for Fusarium head blight resistance in wheat using stochastic simulations. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 21-22. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

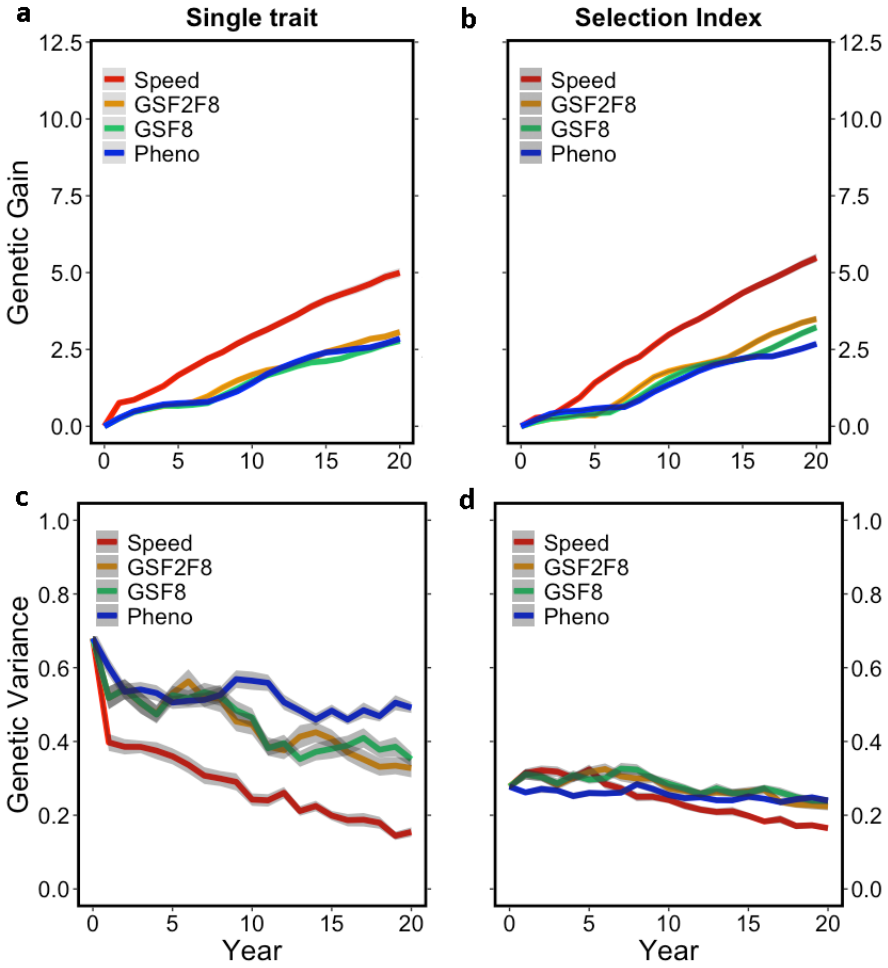

Figure 1 Genetic gain (**a**, **b**) and genetic variance (**c**, **d**) of four simulated breeding schemes in the 'breeding advancement phase' for DON content: **a**, **c** single trait selection approach; **b**, **d** selection index approach.

Keywords

Genetic gain · selection index · single trait selection · speed breeding · *Triticum aestivum*

References and further reading

Gaynor RC, Gorjanc G, Hickey JM (2021) AlphaSimR: an R package for breeding program simulations. G3-Genes Genom Genet 11:jkaa017. DOI: 10.1093/g3journal/jkaa017

Meuwissen THE, Hayes BJ, Goddard ME (2001) Prediction of total genetic value using genome-wide dense marker maps. Genetics 157:1819-1829. DOI: 10.1093/genetics/157.4.1819

Voss-Fels KP, Herzog E, Dreisigacker S, Sukumaran S, Watson A, *et al*. (2019) "SpeedGS" to accelerate genetic gain in spring wheat. In: Miedaner T, Korzun V (eds), Applications of genetic and genomic research in cereals, pp 303-327. Woodhead Publishing, Elsevier. DOI: 10.1016/b978-0-08-102163-7.00014-4

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Looking ahead: Races and resistances to stem rust in European wheat and triticale

Thomas MIEDANER[1], Kerstin FLATH[2], Paul GRUNER[1], Anne-Kristin SCHMITT[2], Philipp SCHULZ[2]

[1] University of Hohenheim, State Plant Breeding Institute, Fruwirthstr. 21, 70599 Stuttgart, Germany

[2] Julius Kühn-Institut (JKI), Institute for Plant Protection in Field Crops and Grassland, Stahnsdorfer Damm 81, 14532 Kleinmachnow, Germany

(✉) miedaner@uni-hohenheim.de

Abstract

Stem rust in wheat caused by *Puccinia graminis* f.sp. *tritici* is an upcoming disease in Europe. Although practically absent in the last five decades, epidemics in Europe have been reported in increasing numbers, *e.g*., 2013 in Germany, since 2016 in Sicily, Italy, 2017 in southern Sweden and 2022 in the United Kingdom. In the RustWatch project, a total of 373 stem rust samples have been collected during 2019-2022 across 19 European countries from Spain to Latvia. Several researchers expect a higher occurrence of stem rust in Europe due to global warming (Prank *et al*., 2019; Miedaner & Juroszek, 2021). Here, we present data on the prevailing stem rust races in Germany and Austria and information on resistances in European wheat and triticale diversity panels and segregating populations.

In Germany and Austria, six distinct genetic groups (clades) have been identified from 56 samples collected during 2017-21 and analysed by twenty SSR (simple sequence repeat) markers. Two clades (Clades III_B, IV_F) are exotic incursions from East Africa and West Asia (pre 2016), clade IV_B is from Ethopia (2013-14, 'Digalu'), the others are from internal spread within the EU. These are the same clades known from other European countries (Patpour *et al*., 2022). Additionally, 79 races were identified from the alternative host barberry (*Berberidaceae*) (Rodriguez-Algaba *et al*., 2022). The results highlight the high mobility of stem rust spores within Eurasia and a fundamental role of barberry in generating new genetic variation.

Resistances in European wheat are scarce. In the RustWatch panel with 263 varieties tested at Berlin-Dahlem and Sicily, Italy, in four years, only 14% of the varieties were resistant (score 1-4 on the 1-9 scale). The correlation between both locations/countries was high across three years ($r = 0.752$, $p < 0.001$) although the experiment in Germany was inoculated with German races and the experiment in Italy was naturally infected. In another diversity panel of 280 winter wheat varieties inoculated by a mixture of German stem rust isolates 14% were highly resistant (0-5% infected stem area) and a further 18% resistant (5-10%) tested across six environments. Molecular analyses of this diversity panel by a 25k iSelect SNP (single-nucleotide polymorphism) chip revealed only three already known major resistance loci for stem rust resistance: *Sr38* on chromosome 2A, *Sr31* on chromosome 1B, and *Sr2*4 on chromosome 3D. All three genes were still effective although none of them provided full resistance (Fig. 1).

These three resistance genes were also detected in different combination in biparental populations with the resistance donors 'Memory', 'LG Character', 'KWS Montana' and two breeding lines. Seven minor QTL have also been additionally found, each explaining 0.6% to 12% of the genotypic variation.

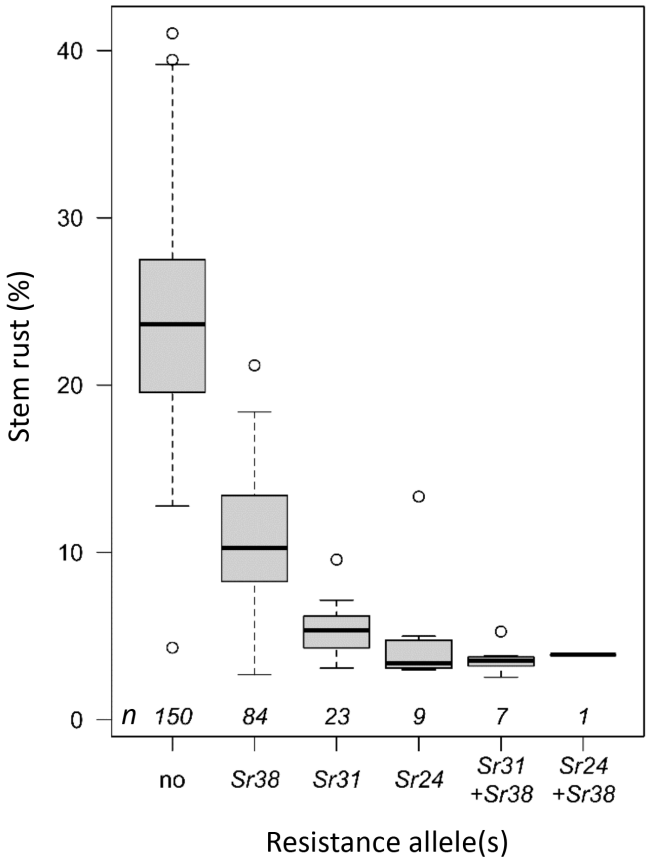

Figure 1 Variation in stem rust severity of genotypes grouped by resistance-linked marker alleles of the respective genes. Number in italics refer to sample size.

Miedaner T, Flath K, Gruner P, Schmitt A-K, Schulz P (2023) Looking ahead: Races and resistances to stem rust in European wheat and triticale. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 23-24. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

A triticale diversity panel of 565 varieties and two biparental populations with 182 and 162 progenies, respectively, was inoculated with a mixture of wheat stem rust isolates. The resistance situation was totally different: the majority of progenies was resistant in all three populations. In the diversity panel five loci have been detected that explained 3.6% to 8.4% of genotypic variation. Four of these loci had already high allele frequencies explaining the high resistance level in triticale. The locus on 3RL segregated also in the DH population Tulus × Massimo explaining 50.1% of genotypic variation and resulting in full resistance. This clearly illustrates that QTL mapping is better suited for the genetic characterization of individual loci. The Cando × Triticon population was monomorphic for the same resistance allele, therefore being fully resistant to stem rust.

In summary, stem rust resistance in European winter wheat is rare as shown by the analysis of two diversity panels with a total of 543 varieties. Only three major genes have been detected in the winter wheat panel with emphasis on German varieties, that are already known from international sortiments. *Sr24*, *Sr31*, and *Sr38* are still effective in Germany, however, in Spain already an isolate from the barberry area has been detected with a combined virulence for all three genes (Patpour *et al*., 2022). Also in Germany, two isolates with each of two combinations of the respective virulences have been detected recently. In future, more efforts should be made by wheat breeders to broaden the basis of stem rust resistance, since it must be assumed that the number and extent of epidemics will increase caused by climate change. Triticale seems to be still very resistant to stem rust.

Keywords

Puccinia graminis · resistance genes · *Triticum aestivum* · *×Triticosecale* · virulences

Acknowledgments

This research was supported by Grant 773311 (RustWatch) of Horizon 2020 and funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany partly via the Federal Office for Agriculture and Food (BLE) under the innovation support program within the project GetreideProtekt (Grant 28-1-B2.021-16)

References and further reading

Bhattacharya S (2017) Deadly new wheat disease threatens Europe's crops. Nature 542:145-146. DOI: 10.1038/nature.2017.21424

Miedaner T, Juroszek P (2021) Climate change will influence disease resistance breeding in wheat in Northwestern Europe. Theor Appl Genet 134:1771-1785. DOI: 10.1007/s00122-021-03807-0

Patpour M, Hovmøller MS, Rodriguez-Algaba J, Randazzo B, Villegas D, *et al*. (2022) Wheat stem rust back in Europe: Diversity, prevalence and impact on host resistance. Front Plant Sci 13:882440. DOI: 10.3389/fpls.2022.882440

Prank M, Kenaley SC, Bergstrom GC, Acevedo M, Mahowald NM (2019) Climate change impacts the spread potential of wheat stem rust, a significant crop disease. Environ Res Lett 14:124053. DOI: 10.1088/1748-9326/ab57de

Rodriguez-Algaba J, Hovmøller M, Schulz P, Hansen JG, Lezáun JA, *et al*. (2022) Stem rust on barberry species in Europe: Host specificities and genetic diversity. Front Genet 13:988031. DOI: 10.3389/fgene.2022.988031

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Mapping of wheat dwarf virus resistance in winter wheat

Maria BUERSTMAYR[1], Hermann BUERSTMAYR[1]

[1] University of Natural Resources and Life Sciences, Vienna, Department of Agrobiotechnology, IFA-Tulln, Institute of Biotechnology in Plant Production, Konrad Lorenz Str. 20, 3430 Tulln an der Donau, Austria

(✉) hermann.buerstmayr@boku.ac.at

Abstract

Wheat dwarf virus (WDV), the causative agent of wheat dwarf disease (WDD), is one of the most damaging viral pathogens in winter wheat. The virus was first documented in 1960 in the former Czechoslovakia (Vacke, 1961). Since then it has spread all over Europe and has been reported in the Middle-East, Western-Asia, China and Africa. Typical symptoms of WDD are dwarfism and lightening or yellowing of leaves, along with reduced or no heading, increased tillering and reduced winter hardiness (Fig. 1). Infection with WDV can cause premature plant death, resulting in high or even complete yield loss. The intensity of damage depends on the time of infection and is highest when infection occurs during the one to three leave stage. Symptoms are milder when infection occurs at later development stages and plants become resistant when first nodes are detectable. The leaf hopper *Psammotettix alienus* (Homoptera: *Cicadellidae*) transmits the virus to its host in a persistent-circulative non-propagative manner. Once the leaf hoppers or nymphs have ingested the virus, they remain infectious throughout their lifetime. However, the virus will not be transferred to the next generation. Primary infection occurs in autumn, when viruliferous leafhoppers infect young plantlets when they are most sensitive. Secondary spread occurs in spring when nymphs hatch, feed on primary infected plants and then transfer the virus to numerous other plants. Leaf hoppers are highly mobile. The activity of the leaf hoppers and their nymphs slows down when the temperature drops below 15°C and stops below 10°C. Their high mobility under warm temperature and the non-availability of approved insecticides make late autumn sowing, when temperatures are low, the only strategy currently available to control the virus. However, late sowing is not effective when long periods of warm temperatures occur towards the end of the year, which is expected to happen more frequently in the future due to climate change. Global warming has already led to an increase in the incidence of WDD, and it is expected to continue to increase further. Growing resistant cultivars would be the preferred measurement to control WDD, but the current wheat cultivars are all susceptible to highly susceptible. Since options to control the disease are very limited, breeding cultivars resitant to WDV is highly recommended. Unfortunately, there is currently almost no information available on host resistance and its genetic regulation. The first and so far only published QTL analysis was performed by Pfrieme *et al*. (2022). They conducted a genome-wide association study (GWAS) using a set of 250 diverse winter wheat accessions and identified 35 putative quantitative trait loci (QTL), of which 14 marker trait associations could be confirmed in bi-parental populations, suggesting a quantitative regulation of resistance to WDV. Considering the increasing importance of WDV, it is necessary to improve the knowledge of the genetic control of WDV disease.

Experimental lines A39 and A40, descending from reciprocal crosses between Austrian winter wheat cultivar 'Capo' and the Dutch experimental line SVP-72017, showed consistent and low WDV symptom severity over three years of pre-testing using early sowing as treatment. The objective of this work was to characterize the genetic architecture of WDV resistance of lines A39 and A40 and to identify QTL that can be used in resistance breeding. A40 or A39 were used as resistant parents and crossed with the susceptible parents 'Midas', 'Mulan' or P1314. Of these crosses four recombinant inbred line (RIL) populations were developed by single seed decent comprising 168, 105, 99 and 130 RILs. RILs and parental lines were evaluated under field conditions for three years at IFA-Tulln, Austria. Natural infestation was provoked by early autumn sowing. WDV symptom severity was visually assessed at two time points in spring resulting in highly reproducible phenotypic data. Genotyping was done using the 7K SNP array provided by TraitGenetics GmbH (Gatersleben, Germany). Multiple QTL mapping was used for QTL analysis.

Two highly significant QTL were identified, one highly efficient on chromosome 6A and one moderately efficient on 1B. The two QTL have an additive effect towards increasing resistance. The QTL on 6A had a strong effect in all experiments and all populations, explaining up to 73.9% of the phenotypic variance. Its resistance originated from donor lines A40 or A39. The QTL on 1B derived its resistance from the susceptible parent P1314 and was identified in both populations that had P1314 as a crossing parent. QTL on 1B explained up to 15.8% of the phenotypic variance and its resistance is putatively associated with the 1RS.1BL translocation present in P1314. Both QTL represent valuable resources for improving WDV resistance in wheat.

Keywords

Buerstmayr M, Buerstmayr H (2023) Mapping of wheat dwarf virus resistance in winter wheat. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 25-26. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

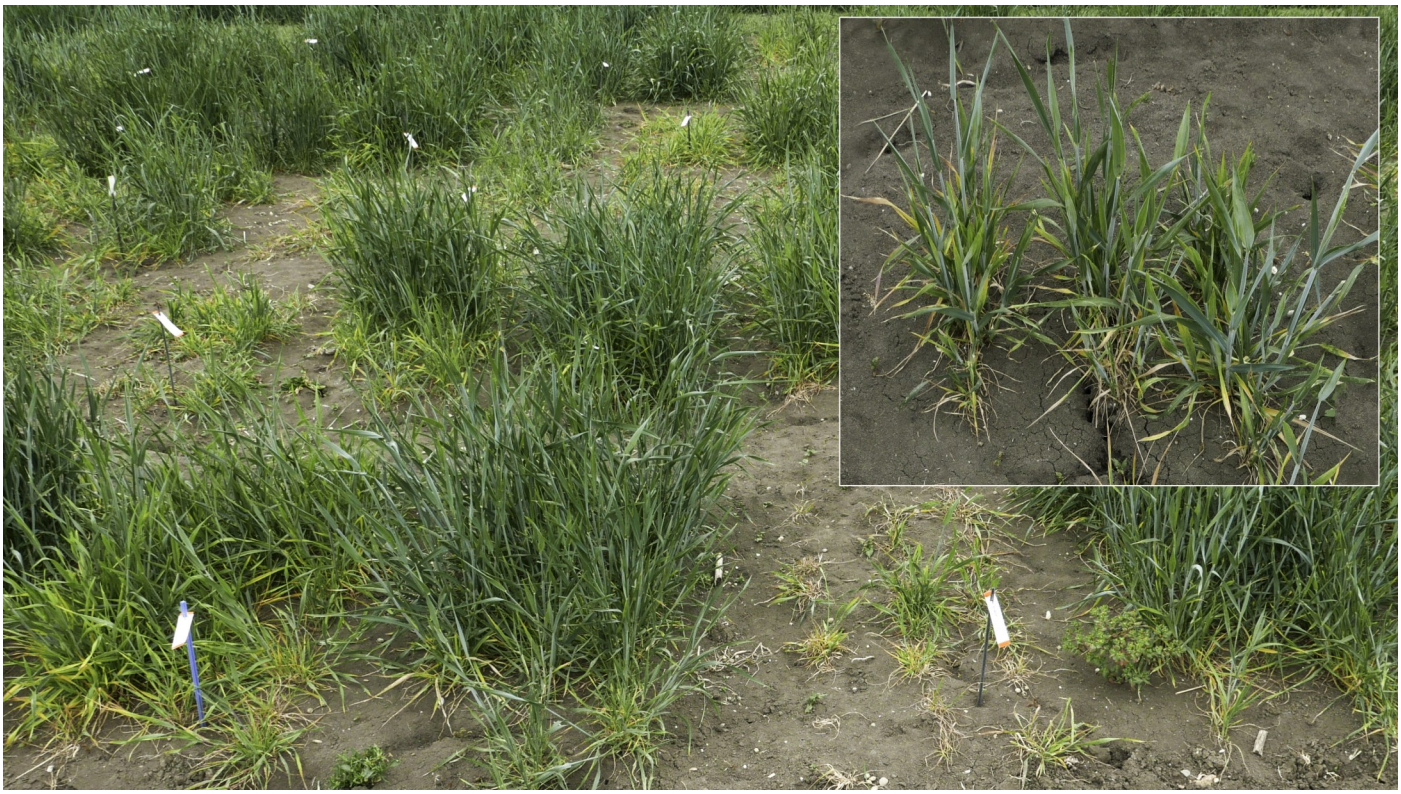

Figure 1 Winter wheat microplots with different intensities of wheat dwarf virus symptoms, *e.g*. chlorosis of leaves (parallel to the veins), reduced fertile tillers, stunted growth (ear often gets stuck in the flag leaf sheath), and dead plants.

Early sowing · leaf hopper · natural infection · QTL mapping · resistance breeding

Acknowledgments

We acknowledge Mathias Fidesser for technical support in the field experiments. Great thanks to Antje Habekuß for performing the DAS-ELISA tests.

Material availability

For seeds of the resistance donors or resistant experimental lines please contact the corresponding author.

References and further reading

Buerstmayr M, Buerstmayr H (2022) Two major quantitative trait loci control wheat dwarf virus resistance in four related winter wheat populations. Theor Appl Genet 136:103. DOI: 10.1007/s00122-023-04349-3

Jones RAC (2021) Global plant virus disease pandemics and epidemics. Plants 10:233. DOI: 10.3390/plants10020233

Kvarnheden A, Lindblad M, Lindsten K, Valkonen JPT (2002) Genetic diversity of Wheat dwarf virus. Arch Virol 147:205-216. DOI: 10.1007/s705-002-8313-x

Lindblad M, Sigvald R (2004) Temporal spread of wheat dwarf virus and mature plant resistance in winter wheat. Crop Prot 23: 229-234. DOI: 10.1016/j.cropro.2003.08.011

Lindblad M, Waern P (2002) Correlation of wheat dwarf incidence to winter wheat cultivation practices. Agric Ecosyst Env 92:115-122. DOI: 10.1016/S0167-8809(01)00302-4

Lindsten K, Lindsten B (1999) Wheat dwarf — an old disease with new outbreaks in Sweden. J Plant Dis Prot 106:325-332.

Pfrieme AK, Ruckwied B, Habekuß A, Will T, Stahl A, *et al*. (2022) Identification and validation of quantitative trait loci for Wheat dwarf virus resistance in wheat (*Triticum* spp.). Front Plant Sci 13:828639. DOI: 10.3389/fpls.2022.828639

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

How long does it take to develop high performing and common bunt resistant winter wheat lines using organics-compliant methods?

Magdalena LUNZER[1], Sebastian MICHEL[1], Maria BUERSTMAYR[1], Heinrich GRAUSGRUBER[2], Hermann BUERSTMAYR[1]

[1] Institute of Biotechnology in Plant Production, Department of Agrobiotechnology, IFA-Tulln, University of Natural Resources and Life Sciences, Vienna, Konrad-Lorenz-Str. 20, 3430 Tulln an der Donau, Austria

[2] Institute of Plant Breeding, Department of Crop Sciences, University of Natural Resources and Life Sciences, Konrad-Lorenz-Str. 24, 3430 Tulln an der Donau, Austria

(✉) magdalena.lunzer@boku.ac.at

Abstract

Once among the most devastating wheat diseases, common bunt caused by *Tilletia tritici* and *T. laevis* was successfully banned from most fields by the invention of seed dressings with hexachlorobenzenes (HCBs) in the 1950s. During the past decades, a continuously increasing area of agricultural land has been converted to organic management, refraining from the use of chemical pesticide applications. Therefore, common bunt as a primarily seed-borne disease is experiencing a come-back since no alternative and equally effective treatments to seed dressings are available. The most sustainable and efficient way to avoid yield and quality losses due to bunt infections is the use of resistant cultivars. Although 17 different resistance genes have been postulated so far, only few have been mapped and are available for applied breeding. In consequence, the development of bunt resistant cultivars is slow and a small number of varieties with high resistance levels are currently available. In this study, we therefore aim to determine how fast breeding lines can be selected that unite bunt resistance and good agronomic performance.

For this purpose, we developed pseudo-back-cross populations with bunt resistance alleles introgressed from exotic donor lines. Resistance QTL in these donors were mapped in previous projects at IFA-Tulln, enabling marker-assisted selection (MAS) via KASP-markers (Muellner *et al.*, 2020; 2021). The three resistance donors 'Blizzard', 'Bonneville' (US cultivars registered in the 1990s) and PI 119333 (differential line for the bunt resistance gene *Bt12*) were initially crossed to the susceptible cultivar 'Rainer'. During population development, three back-crossing steps were carried out, each with a different back-crossing parent that was either a variety or an advanced breeding line adapted to Austrian growing conditions. After each back-crossing step, the F_1-progeny was screened for the presence of one to three different resistance QTL inherited from the donors using KASP-markers. In generation BC_3F_1, the number of lines was reduced further by one step of genomics-assisted selection (GAS) based on genomic estimated breeding values (GEBVs), filtering out those lines with promising genetic backgrounds based on genome-wide marker data from genotyping by sequencing (GBS). After the last back-cross, the selected progenies were self-pollinated to generate lines harbouring the resistance QTL fixed in a homozygous allelic state. These lines were identified with another round of MAS. Only the selected homozygous resistant lines were subsequently subjected to field tests for common bunt resistance as well as for yield and quality traits together with a control panel of negatively selected lines. Data from two seasons of common bunt testing in artificially inoculated field trials in Austria and one season of dwarf bunt testing with artificial inoculation in Utah (USA) is available to determine disease resistance levels in the population. In addition, a replicated yield trial was conducted in 2022.

The number of lines undergoing propagation in the greenhouse or field testing was greatly reduced by the MAS and GAS steps. After the individual selection steps in each of the three back-cross generations, 33.6%, 8.8% and 9.1% respectively, of all lines were chosen to be kept in the population. Thereby, not only resources required for field testing were kept low, but also the time from the initial cross to the first homozygous resistant lines in generation BC_3F_2 was reduced by more than 50% compared to a selection scheme based solely on phenotypic selection. Of all lines selected to harbour one or several of the introgressed resistance QTL, 35% (69 lines) were fully or highly resistant (≤5% incidence) to common bunt across two years. Several factors contribute to the fact that almost two thirds of the population showed mild to severe infections: markers applied for MAS were not diagnostic but only flanking the chromosomal regions conferring resistance. The complex pedigrees with five different parents for each line led to a loss of polymorphic markers with each back-crossing step. Individual loci could therefore be selected with only a single marker in some of the lines, leading to low selection accuracies. In addition, some of the resistance loci conferred by the donor lines do not provide full resistance on their own but only in combination with a second locus. As some of the intervals flanked by the applied markers are relatively large, also recombination

Lunzer M, Michel S, Buerstmayr M, Grausgruber H, Buerstmayr H (2023) How long does it take to develop high performing and common bunt resistant winter wheat lines using organics-compliant methods? In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 27-28. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

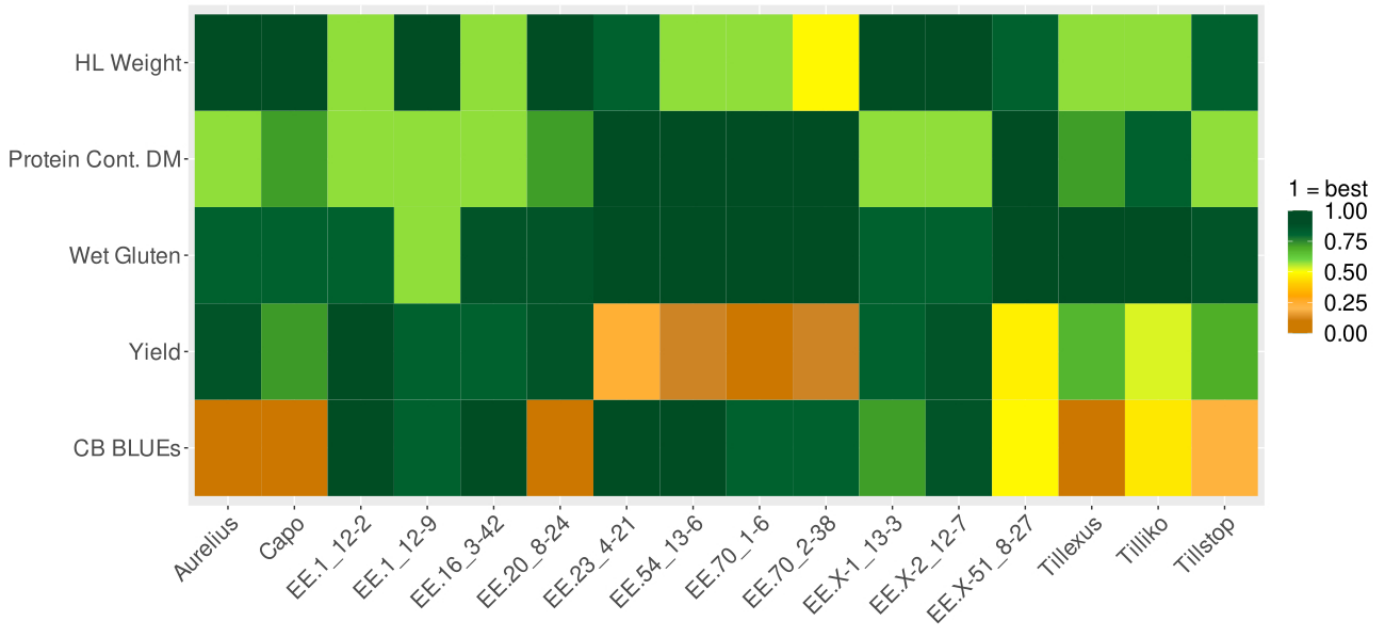

Figure 1 Heatmap showing scores for hectolitre weight, protein content, wet gluten content, yield and common bunt infections across two seasons normalized to a range between 0 and 1 with 1 being the best, desired value (*e.g.*, no bunt infection and high yield both have a score of 1). Scores for quality traits were allocated by considering Austrian thresholds for different wheat quality classes in trading. Scores are given for two bunt-susceptible check cultivars (*i.e.*, 'Aurelius' and 'Capo'), the six best-performing experimental lines in terms of yield and the five best-peforming lines in terms of protein content (genotype names with the prefix "EE"), as well as three cultivars originally registered as bunt-tolerant in Austria (*i.e.*, 'Tillexus', 'Tilliko' and 'Tillstop'). Data on all traits except common bunt is from replicated field trials conducted in Tulln in 2022. Data on common bunt incidence are shown as best linear unbiased estimates (BLUEs) across 2021 and 2022.

events might have occurred in these regions that could not be tracked with the markers and that led to a loss of resistance in positively selected lines.

We also observed that common and dwarf bunt resistance are not conferred by the same genes in our experimental population. While lines harbouring the resistance locus on chromosome 1B showed high resistance against common bunt, they were to a large extent infected by dwarf bunt. The opposite pattern was observed for lines with the *Bt12*-locus on chromosome 7D where most likely recombination events in the chromosomal region were responsible for a loss of resistance against common bunt but not against dwarf bunt. Common bunt incidence was uncorrelated with yield and quality traits. We found experimental lines with complete resistance against common bunt that performed equally well or slightly better in terms of yield and quality than the highly susceptible check cultivars (Fig. 1). Cultivars registered as bunt tolerant in Austria and Germany that were included were moderately to highly infected with common bunt in our trials.

We therefore conclude that MAS is a suitable method to reduce time and resources for the development of bunt resistant and high -performing winter wheat lines. The experimental lines in our population were tested in generation BC_3F_{2n}. Using MAS, it is possible to reach this generation in 2.5 years, while selecting exclusively via phenotypes would take 5.5 years for the same outcome and require a lot of additional resources.

Keywords

Marker-assisted selection · organic agriculture · resistance breeding · seedborne disease · *Tilletia* · *Triticum aestivum*

Acknowledgements

M. Ehn is recipient of a DOC Fellowship of the Austrian Academy of Sciences (Grant nr. 25453). This work was supported by the Doctoral School AgriGenomics of the University of Natural Resources and Life Sciences, Vienna. This work received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 771367 (ECOBREED)

References and further reading

Ehn M, Michel S, Morales L, Gordon T, Dallinger HG, *et al*. (2022) Genome-wide association mapping identifies common bunt (*Tilletia caries*) resistance loci in bread wheat (*Triticum aestivum*) accessions of the USDA National Small Grains Collection. Theor Appl Genet 135:3103-3115. DOI: 10.1007/s00122-022-04171-3

IFOAM - Organics International (2017) Position paper: Compatibility of breeding techniques in organic systems. IFOAM, Bonn. [https://www.ifoam.bio/compatibility-breeding-techniques-organic-systems; accessed 21 March 2023]

Muellner AE, Buerstmayr M, Eshonkulov B, Hole D, Michel S, *et al*. (2021) Comparative mapping and validation of multiple disease resistance QTL for simultaneously controlling common and dwarf bunt in bread wheat. Theor Appl Genet 134:489-503. DOI: 10.1007/s00122-020-03708-8

Muellner AE, Eshonkulov B, Hagenguth J, Pachler B, Michel S, *et al*. (2020) Genetic mapping of the common and dwarf bunt resistance gene *Bt12* descending from the wheat landrace PI119333. Euphytica 216:83. DOI: 10.1007/s10681-020-02614-w

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Improving overwintering in times of climate change - A GWAS for late-frost tolerance of winter faba bean

Alex WINDHORST[1], Cathrine KIEL SKOVBJERG[2,3], Stig UGGERHØJ ANDERSEN[4], Wolfgang LINK[1]

[1] Department of Plant Breeding Methodology, DNPW, Georg-August-University Göttingen, Carl-Sprengel-Weg 1, 37073 Göttingen, Germany

[2] Center for Quantitative Genetics and Genomics, Aarhus University, C. F. Møllers Allé 3, bld. 1130, 8000 Aarhus, Denmark

[3] Nordic Seed A/S, 8300 Odder, Denmark

[4] Department of Molecular Biology and Genetics, Aarhus University, Universitetsbyen 81, 8000 Aarhus, Denmark

(✉) alex.windhorst@uni-goettingen.de

Abstract

Winter faba bean (*Vicia faba* L.) breeding gains in importance; the interest in faba bean as high protein grain legume crop is continuously increasing across Europe. Currently, the lack of winter hardiness is a major constraint of winter faba bean cultivation in central and northern European countries. One substantial aspect of winter hardiness is tolerance against late-frost, that is frost spells in early spring when plants have lost their cold acclimation (are dehardened). In this study, we present the first genome-wide association study (GWAS) on late-frost tolerance in winter faba bean. We phenotyped a panel of 188 inbred lines as juvenile plants in controlled experiments. Three sub-traits ($0.65 < h^2 < 0.81$) representing both the direct damage and the subsequent effects of late-frost stress were assessed. Utilizing 17,211 high-quality SNPs, we identified two, three, and nine significant marker-trait associations per sub-trait, respectively. Among them, large effect putative QTL explaining up to 22.93% of the observed sub-trait phenotypic variance were found. Taken per sub-trait, the sum of significant markers explained about 32.33%, 36.47%, and 44.92% of the observed phenotypic variance. Two chromosomal regions were found to harbor putative QTL with possible pleiotropic effects on at least two of three sub-traits. Our results could be employed in winter faba bean breeding programs to accelerate breeding progress towards an increased winter hardiness by marker assisted selection (MAS).

Keywords

Abiotic stress · breeding · genome-wide association study · marker score · *Vicia faba*

Introduction

The interest in faba bean (*Vicia faba* L.) has increased in the past decade in many European countries. Its high seed protein content of ≈30% makes faba bean a valuable grain legume crop for animal nutrition and human consumption. Germany's faba bean acreage increased from 16,500 ha in 2013 to 71,100 ha in 2022 (FAOSTAT, 2022; Statistisches Bundesamt, 2022). Except for the UK and France, the cultivation of faba bean, however, is limited to spring-type cultivars (Sass, 2022). In the UK, ≈40 % of the total faba bean acreage (188,000 ha) was cultivated with winter faba bean in 2022. In France, this proportion increased recently to ≈75% of the total acreage of 68,410 ha (Sass, 2022). Facing climate change with consistently increasing temperatures and drought periods during summer, substantial yield losses in spring-type cultivars become more frequent. Winter-type cultivars, in contrast, can partially escape summer droughts (Link *et al*., 2010). In addition, autumn sowing enables a longer growing period and better use of moisture during early spring (Link *et al*., 2010). The plants thus tend to be more robust against pests and pathogens in spring. However, the main disadvantage of winter faba bean is their limited winter hardiness. The risk of winter-kill is a major constraint that currently prevents the large-scale cultivation of winter faba bean in central and northern Europe. Especially, the tolerance against frost and late-frost, the latter occurring in spring, gains more importance considering the unpredictable temperature fluctuations due to climate change. Milder autumn temperatures reportedly were already affecting the cold acclimation of pea and other grain legumes (Castel *et al*., 2017). Likewise, winter faba bean could be affected and thus be less tolerant to freezing temperatures during winter (Carrillo-Perdomo *et al*., 2022) and after winter. Frost tolerance in winter faba bean has been studied extensively with many different approaches (for review see Link *et al*., 2010). At the University of Göttingen, for example, a sophisticated frost stress protocol, based on climate chamber experiments, was developed since 2003 (Arbaoui *et a*l.,

Windhorst A, Kiel Skovbjerg C, Uggerhøj Andersen S, Link W (2023) Improving overwintering in times of climate change - A GWAS for late-frost tolerance of winter faba bean. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 29-36. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

2008; Arbaoui & Link, 2008; Roth & Link, 2010; Sallam *et al*., 2015; Ali *et al*., 2016). In contrast, no research on late-frost tolerance in juvenile winter faba bean plants (only on *e.g*., detached leaves) was published so far. To investigate this trait, we adapted the frost stress protocol to apply late-frost on fully dehardened plants and assessed multiple sub-traits of late-frost tolerance. A genome-wide association study (GWAS) was conducted in a winter faba bean inbred line panel. The aim was to identify late-frost tolerance QTL for subsequent marker development and application in winter faba bean breeding.

Material and methods

Plant material

In this study, 188 winter faba bean inbred lines were used, in the following referred to as association-set (A-set). The inbred lines are derived from the Göttingen Winter Bean Population (GWBP) via single-seed descent for more than nine generations ($F_{>9}$). In 1989, the GWBP was created by combining 11 founder lines into a Syn-0 like mixture. Ali *et al*. (2016) described the founder lines as Hiverna/1, Webo/1, Wibo/1, L79/79/1, L977/88/S1wn, L979/S1/1/1sn (German lines), Banner/1, Bourdon/1, and Bulldog/1 (UK lines), and Arrissot and Côte d'Or/1 (French lines). For eight generations, the population reproduced via its natural, partial allogamous reproductive mode with about 50-60% outcrossing (Gasim *et al*., 2004) and no artificial selection was employed. The single-seed descent procedure to develop the A-set lines started there right after (Gasim, 2003).

In addition to the A-set, 24 winter faba bean and four spring faba bean inbred lines were used as checks. These inbred lines are derived from former cultivars or in-house crosses, including the above-mentioned founder lines. The total test panel consisted of 216 inbred lines (entries).

Phenotyping

To assess late-frost tolerance, an experimental series was run between September and April in 2020-2021 and 2021-2022. Juvenile plants were tested in a plant growth chamber under controlled environment conditions. Five experiments with two replications each were conducted, yielding in total 10 replicates. Following an α-lattice design, the 216 entries were assigned to 18 incomplete blocks with 12 entries each. An incomplete block was defined as three pots (size 17 × 17 cm^2) with four entries per pot and two plants per entry. Thus, each replicate consisted of 54 pots (covering 4 m^2). The 4 m^2 plant growth chamber (Vötsch VB4018) was equipped to provide 200 µmol s^{-1} m^{-1} light and regulate temperatures down to -20°C.

The seeds were sown following a strict protocol to control sowing depth and position within the pot. Local compost and sand (3:1 mixture) were used. Soil water content was gravimetrically controlled to be at ≈70-80% throughout the experiments. Germination took place in mild greenhouse conditions (10-15°C, 12 h light). At about two-leaf stage, plants were allocated to the growth chamber for a 10-d hardening at 5°C/2°C (day/night) followed by 10 d of so-called winter at 3°C/-1°C, both with a 10 h photoperiod. Afterwards, the plants were dehardened in the greenhouse at 7°C and 12-h photoperiod for 10 days. This time period was shown to be sufficient to ensure almost total loss of the cold acclimation (Herzog, 1989). Hence, dehardened plants were obtained by this protocol. For the subsequent frost test, the pots were placed in boxes made from Styrodur® to insulate roots from severe freezing damages. In three successive nights, freezing temperatures of -13°C, -15°C, and -17°C were applied, respectively. The freezing level was reached after 4 h of linear temperature decline. It was maintained for 4 h followed by the linear return to mild day temperature of 5°C within 6 h (Ali *et al*., 2016; Arbaoui *et al*., 2008; Arbaoui & Link, 2008; Roth & Link, 2010; Sallam *et al*., 2015). The air humidity was not controlled. After the frost test, the plants were transferred back into the greenhouse to first get 4 d of recovery followed by 30 d regrowth phase at mild temperatures (15°C/10°C) and a 12 h photoperiod.

A total of 19 different traits and freezing-related sub-traits were assessed. We here report three sub-traits chosen to jointly describe the trait late-frost tolerance: (i) loss of turgidity and color (LossTC), (ii) disposition to survive (DtS), and (iii) regrowth (REG). LossTC was calculated from the loss of leaf turgidity (scale 1-9, with 9 representing the greatest loss) and the loss of leaf color (scale 1-9, with 1 representing no color change and 9 representing black). The loss of leaf turgidity as well as the loss of leaf color were scored on each individual plant 10 h after each freezing night and again after the 4 d of recovery, respectively. These eight scores were summed up to form the combined sub-trait LossTC (scale 16-64). DtS was assessed during the regrowth phase. To enter the regrowth phase, the main stem and tillers of all plants were chopped off directly above the second node. In addition, all unfolded leaves were removed. In the 30 days of regrowth, a daily assessment of whether a plant was alive or dead was performed. The number of days a plant survived within the regrowth phase was transformed into the DtS (0° to 90°): $\arctan(x_i/\mu_x)$, with x_i = number of days until death and μ_x = average number of days until death for those plants that died within the regrowth phase. The surviving plants were scored as 90° (Roth & Link, 2010; Sallam *et al*., 2015). The complete shoot matter fresh weight of the survival plants was measured in grams. The mean regrowth of both plants per entry was taken as REG (g).

The phenotypic data of these three sub-traits was first analyzed according to the α-lattice design of the five experiments. The lattice-adjusted means of the five experiments (five lattices) were utilized to analyze this series of experiments with "genotypes" as random effect and "experiments" as fixed effect using PLABSTAT (Utz, 2011). The phenotypic means of the genotypes across the E = 5 experiments per sub-trait were utilized in the genome-wide association study. Repeatability (h^2) was calculated for E = 5 as:

$$h^2 = \frac{variance\ component\ of\ genotypes}{variance\ component\ of\ genotypes\ +\ \frac{1}{5}MS(e)}$$

SNP genotyping

The A-set was genotyped with the Vfaba_v2 Axiom SNP array containing approximately 60K probes (Khazaei *et al*., 2021; O'Sullivan, 2019). A detailed description of the DNA extraction process, quality control, filtering of the genotyping data and alignment of high-quality SNP to the *V. fa*ba reference sequence (Jayakodi *et al*., 2023; https://projects.au.dk/fabagenome/genomics-data) was given in Skovbjerg *et al*. (2023). In addition, the faba bean genome consortium has subdivided the very large faba bean chromosome 1 into two parts (Chr 1S and Chr 1L) at

position 1,574,527,093 to facilitate data analysis (Skovbjerg *et al*., 2023). Thus, chromosome 1 will be displayed as Chr 1S and Chr 1L in the following. Of the final set of 21,345 physically mapped high-quality SNPs, 19,060 SNPs were polymorph in the A-set. The polymorphic set of SNPs was filtered as follows: First, SNPs with missing data in ≥10% of the inbred lines were discarded. Second, SNPs with a heterozygosity level of ≥10% were removed. Third, SNPs with a minor allele count (MAC) of <10 were removed. Finally, all missing SNP data was imputed with the LD-kNNi algorithm in TASSEL 5 (Bradbury *et al*., 2007; Money *et al*., 2015). The final set of 17,211 SNPs was used for further downstream analyses.

Genome-wide association study

GWAS was performed using the Bayesian-information and Linkage -disequilibrium Iteratively Nested Keyway (BLINK) implemented in the GAPIT v. 3 library implemented in R software (Huang *et al*., 2019; Wang & Zhang, 2021). The first three principle components (PCs) were used as covariates to prevent signals from possible population stratification. The absence of *p*-value inflation was checked by visual inspection of the respective Q-Q plots (data not shown). Significances were tested with the false discovery rate (FDR) method of Benjamini & Hochberg (1995) by applying an FDR threshold of 5%. Effect size and phenotypic variance explained (PVE in %) of each significantly associated marker is reported as provided by BLINK output. To check for correlation between the GWAS results of sub-traits, we first assigned a rank position to each SNP according to its *p*-value. Subsequently, the Spearman's rank correlation coefficient was calculated for each pair of sub-traits based on these SNPs rank position.

Marker scores

Marker scores for each sub-trait were calculated based on the effect sizes of its significant markers. The effect sizes were added up for each A-set inbred line according to the respective marker genotype. A beneficial allele could either reduce or increase the phenotypic value depending on the sub-trait's nature, that is, for LossTC (scale points) a beneficial allele reduces the total score while for DtS (°) and REG (g) it increases the total phenotypic value. The total sum, *i.e*., the marker score, predicts the performance of the genotype, thus it was correlated to the phenotypic data of the inbred lines used for GWAS. The squared correlation, *i.e*., coefficient of determination (R^2) reported, is hence estimating the proportion of explained phenotypic variance when using all significant markers jointly in a multiple regression analysis.

Linkage Disequilibrium

Linkage Disequilibrium (LD) was estimated for the A-set using PLINK v. 1.9 (Purcell *et al*., 2007). To this end, the squared correlation coefficient (R^2) was computed chromosome-wise for each pair of SNPs. LD decay along increasing distances (bp) between marker loci was inspected; to that end, the resulting LD data was sorted by SNP-pair bp-distance, and binned into groups of 1000 data points (distances). Subsequently, the average R^2 was plotted against the average distance per each bin. A smoothed curve was fitted using the loess function with a 10% smoothing span in R. This was done first chromosome-wise and second genome-wide. For the latter, the resulting LD data was first merged across chromosomes beforehand the downstream analysis was conducted. The distance (bp) where the fitted LD curve reached half of its maximum was defined as LD decay region, either genome-wide or chromosome-wise (Skovbjerg *et al*., 2023).

Candidate gene search

The physical position of a marker was searched in the *V. faba* gene annotation (Jayakodi *et al*., 2023). Both the coding sequence (CDS) and the protein sequence were subjected to the blastn and blastp function of NCBI BLAST, respectively. In blastn search settings, *Medicago truncatula* was specified as organism. Additional information referring to the BLAST output was searched at Arabidopsis Information Resource (TAIR; Berardini *et al*., 2015) and Ensembl Genomes (Yates *et al*., 2022) using the *M. truncatula* (MedtrA17_4.0) reference genome.

Results and discussion

Several prior studies reported on frost tolerance of winter faba bean inbred lines (Ali *et al*., 2016; Arbaoui & Link, 2008; Roth & Link, 2010; Sallam, 2014). The A-set or subsets of it were phenotyped for multiple sub-traits of frost tolerance in an experimental set-up comparable to ours. Ali *et al*. (2016) reported the first genome-wide association study in winter faba bean for an abiotic stress tolerance, *i.e*., frost tolerance. In contrast to that study, we here report the first genome-wide association study in winter faba bean for late-frost tolerance. With the conducted experimental series of 10 replicates, we found an equally high h^2 for the two sub-traits LossTC (81.18%) and DtS (81.43%). REG, however, had a lower h^2 of 64.65% (Table 1). REG is the sub-trait that was affected the most by uncontrolled environmental conditions, such as day length and temperature. Within the regrowth phase the plants were kept in a greenhouse without air-conditioning. Hence, unusual high day temperatures during the winters 2020-21 and 2021-22 (>15 °C) favored a strong regrowth for some of the replicates. Furthermore, the difference in day length during the winter months vs. the spring months of this series of experiments might have affected the regrowth as well. Replicates that entered the regrowth phase in winter were kept at 12 h artificial light, strictly; whereas replicates that entered the regrowth phase in spring (end of February – March) might have benefited from the then-stronger natural sunlight and an increased sunlight period. Taken together, this might explain the lower h^2 for REG as compared to LossTC and DtS.

Table 1 Phenotypic results of late-frost tolerance sub-traits (*i.e*., loss of turgor and color LossTC, disposition to survive DtS, and regrowth REG) after five experiments (10 replicates).

Sub-trait	Mean	Min	Max	SD	h^2	$LSD_{0.05}$
LossTC (scale points)	31.14	28.11	42.48	2.83	81.18	3.50
DtS (°)	63.62	24.62	89.71	12.55	81.43	14.89
REG (g)	1.98	0.04	4.42	0.95	64.65	1.56

Max, maximum value; Min, minimum value; SD, standard deviation; LSD, least significant difference (α=0.05)

Analysis of variance for all three sub-traits among the A-set lines displayed highly significant variation (F value, p <0.01) due to genotype and due to experiment (details not given). The sub-trait LossTC was significantly (p <0.01) negatively correlated with DtS (r = -0.72) and REG (r = -0.56, Fig. 1). The strong negative correlation was to be expected, since a great loss of turgidity and color represents severe frost damage. Plants showing such severe frost damage are hence less likely to survive and recover in the following weeks. The DtS and REG, in contrast, were strongly positively correlated with r = 0.73 (p <0.01, Fig. 1) indicating a positive genetic relationship between those sub-traits.

For the association analysis, a final set of 17,211 SNPs and 183 A-set lines were used. Five inbred lines were excluded from the A-set based on doubtful genotyping results. Their supposed levels of heterozygosity were strikingly higher (>5%) than in the A-set on average (1.72%) and did thus not meet the homozygosity level as expected at $F_{>9}$. Taking the total genome size of *V. faba* of ≈13 Gb into account (Khazaei *et al.*, 2021), we had a genome-wide average SNP density of ≈1 SNP/Mb. Based on the SNP set, a genome-wide LD decay region of 1,321,923.9 Mb was calculated (data not shown). The theoretical SNP density fitted the actual SNP density per Mb window size throughout the major proportion of the genome (data not shown). Thus, the utilized SNP set was considered sufficient to detect QTL across the genome within the given A-set. Correspondingly, we found significant marker-trait associations for all sub-traits of late-frost tolerance. For LossTC, one marker on the short arm of chromosome 1 (Chr 1S) and two on Chr 4 passed the FDR threshold (Fig. 2, Table 2). In total, they added up to 36.47% PVE of which marker 2 (AX-416762439, 22.9% PVE) on Chr 4 contributed the major proportion. With an effect size of 1.83 scale points marker 2 had the largest effect among the detected markers as well. Its beneficial allele "T" was rather rare with an allele frequency of 7.1%, which equals 13 out of 183 A-set lines. Moreover, the "T" allele effect reduced the LossTC scoring value by about 12.73% of the observed total range of the phenotypic values for LossTC among the A-set. Especially for DtS, the association analysis yielded multiple significant markers on four of the displayed chromosomes (Fig. 2, Table 2). Three markers (4, 5, and 11) were found on Chr 2 with two of them (marker 4 and 5) having the strongest signals found among all nine markers. Their effect sizes, however, differed greatly with 4.12° in case of AX-181492673 and 12.13° for AX-416781916. The latter, marker 5, had the largest PVE of 16.97%, whereas the marker 4 had only 2.06% PVE. The beneficial allele "T" of marker 5 (AX-416781916) was the major allele with an allele frequency of 96.99 % (= 177 lines homozygous and one line heterozygous at the marker locus) and is thus close to be fixed in the A-set. Considering the effect size, the absence of the beneficial allele reduced a line's DtS by 18.64% of the observed total range of the phenotypic values in the A-set. For the marker 4 (AX-181492673), however, 122 A-set lines are homozygous for the beneficial allele "C", which equals an allele frequency of 66.66%. Another noteworthy marker is marker 8 (AX-181481977) on Chr 5. Ranked as fifth marker with respect to the p-value, it still had both the second largest effect size (4.56°) and PVE (12.44%). At this marker locus, 27 A-set lines are homozygous for the beneficial allele "T" and one line is heterozygous, which equals an allele frequency of 15.03%. The remaining six markers ranged between 1.21% and 3.15% PVE with effect sizes of 2.38° to 8.04°. In summary, all nine markers together explained 44.92% of the observed phenotypic

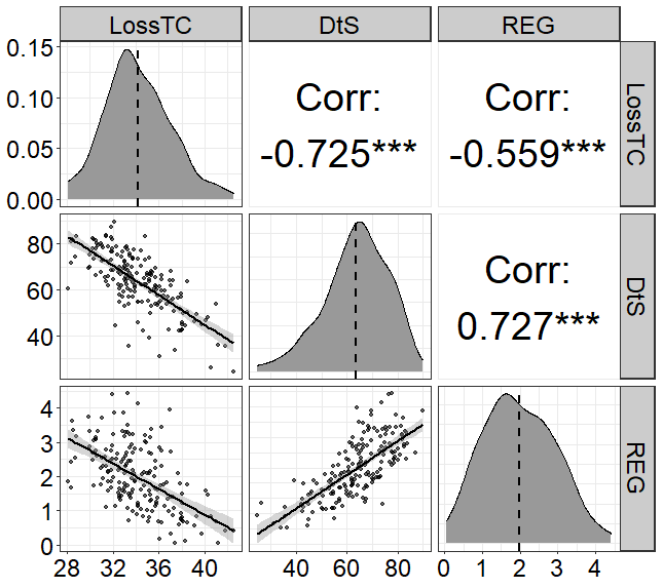

Figure 1 Summary of phenotypic results for the sub-traits loss of turgor and color (LossTC, in scale points), disposition to survive (DtS, in °), and regrowth (REG, in g) in the A-set (n = 183 inbred lines). Above diagonal: correlation coefficients per sub-trait pair (*** = p <0.001); diagonal: density plot per sub-trait with indicated mean as dashed line; below diagonal: correlation plots for sub-trait pairs and linear regression lines; R^2 results: LossTC and DtS (R^2 = 0.53); LossTC and REG (R^2 = 0.31); DtS and REG (R^2 = 0.53).

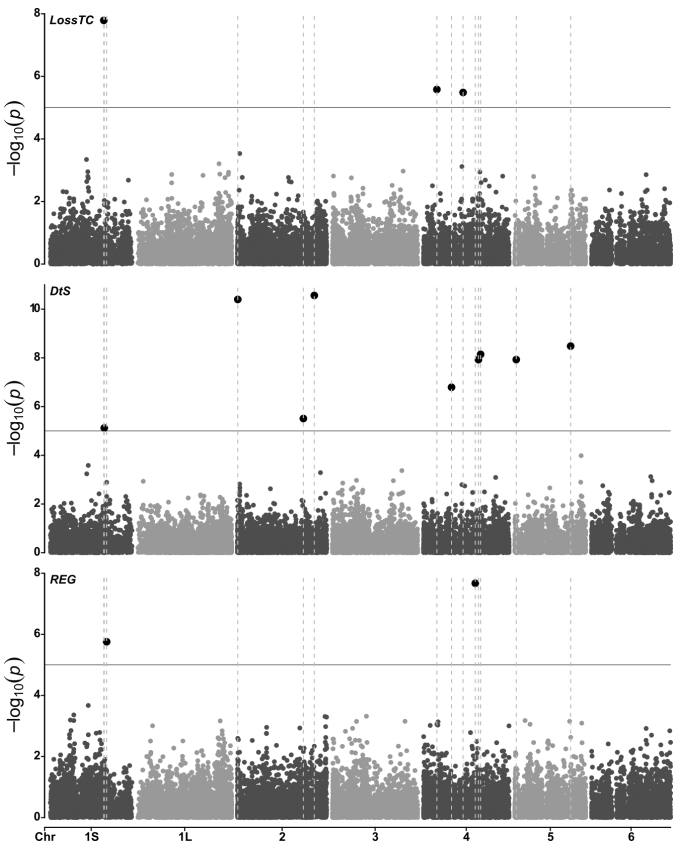

Figure 2 GWAS results as Manhattan plots for the sub-traits loss of turgor and color (LossTC), disposition to survive (DtS), and regrowth (REG). Position of significant marker indicated by vertical dashed line across Manhattan plots. Significance threshold at FDR of 5% in the set of 17,211 SNPs.

Table 2 Genome-wide association analysis results of late-frost tolerance sub-traits. Loss of turgor and color (LossTC); disposition to survive (DtS); regrowth (REG); chromosome number (Chr); physical position on chromosome (Pos); phenotypic variance explained (PVE); beneficial allele (BenAll); allele frequency of beneficial allele (Freq).

Sub-trait	No.	Marker	Chr	Pos	*p*-Value	Effect	PVE (%)	BenAll	Freq
LossTC	1	AX-416808662	1S	1,026,313,397	1.63 e^{-08}	1.00	6.53	A	0.35
	2	AX-416762439	4	257,042,711	2.63 e^{-06}	1.83	22.93	T	0.07
	3	AX-181184990	4	761,474,519	3.27 e^{-06}	1.01	7.24	C	0.82
DtS	4	AX-181492673	2	1,477,896,923	2.74 e^{-11}	4.12	2.06	C	0.67
	5	AX-416781916	2	5,990,977	4.00 e^{-11}	12.12	16.97	T	0.97
	6	AX-181488114	5	1,073,217,918	3.30 e^{-09}	3.98	1.21	G	0.36
	7	AX-416809725	4	1,099,089,179	7.16 e^{-09}	3.56	3.04	G	0.38
	8	AX-181481977	5	26,650,791	1.18 e^{-08}	4.56	12.44	T	0.15
	9	AX-416774047	4	1,056,618,259	1.19 e^{-08}	3.84	1.60	C	0.78
	10	AX-416743268	4	538,907,812	1.61 e^{-07}	3.16	1.89	T	0.30
	11	AX-416773936	2	1,267,663,107	3.09 e^{-06}	8.04	3.15	G	0.97
	12	AX-416790972	1S	1,035,152,021	7.55 e^{-06}	2.38	2.56	G	0.37
REG	13	AX-181193117	4	995,596,193	2.13 e^{-08}	0.34	10.37	G	0.46
	14	AX-416775843	1S	1,081,824,389	1.75 e^{-06}	0.62	21.96	C	0.05

variance for DtS. For REG, however, only two markers were found to be significantly associated. They were located on Chr 4 and Chr 1S, respectively (Fig. 2, Table 2). The effect size of marker 13 (AX-181193117) was only about half as large as of the marker 14 (AX-416775843; 0.34 g vs. 0.62 g). The same holds true for the PVE with 10.37% and 21.96%, respectively. Thus, the two markers added up to 32.33% PVE. For both markers the beneficial allele ("G" and "C") was the minor allele with allele frequencies of 46.45% and 5.19%. The beneficial allele of AX-416775843, for example, increased a line's REG by 14.61% of the observed total range of the phenotypic values in the A-set.

Considering the high correlations among the sub-traits, significant marker-trait associations at the same marker locus for at least two of three sub-traits were expected. However, we did not find any marker meeting that expectation. As Fig. 3 shows, neither of the markers significant for one sub-trait was even close to be significant for any other sub-trait. Moreover, the Spearman's rank correlation between GWAS results among all sub-traits did not exceed $r_s = 0.15$. The obvious assumption that frequently a significant SNP for one sub-trait only narrowly missed significance for a second sub-trait was not supported by the data. However, there were two chromosomal regions that seemed to harbor one or more putative QTL for at least two of the sub-traits. The first region was located on Chr 1S with a marker for LossTC, DtS, and REG in the range of 1,026,313,397 bp and 1,081,824,389 bp (Fig. 2). Thus, the markers AX-416808662 (LossTC) and AX-416790972 (DtS) were not only physically close but also in a moderate LD of $r^2 = 0.15$ exceeding the LD average ($r^2 = 1.43e^{-2}$) of Chr 1S. In addition, their physical distance of 8,838,624 bp was within the range of the chromosome-wise LD decay region (27,486,128.83 bp) for Chr 1S. The marker for REG (AX-416775843), however, was neither in a LD with AX-416808662 ($r^2 = 8.65e^{-5}$, LossTC) nor AX-416790972 ($r^2 = 0.01$, DtS). Furthermore, the physical distance to the DtS marker (46,672,368 bp) was quite large and beyond the chromosome-wise LD decay region of Chr 1S. Therefore, one putative QTL with pleiotropic effect on at least LossTC and DtS could be inferred.

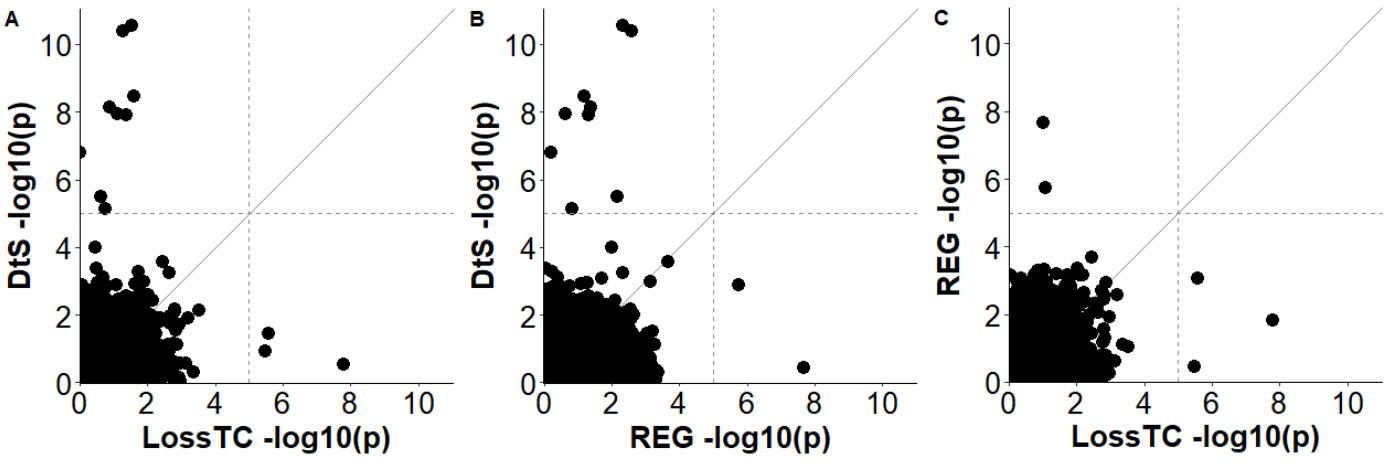

Figure 3 GWAS results as -$\log_{10}$(*p*-value) scatterplot per sub-trait pair of the sub-traits loss of turgor and color (LossTC), disposition to survive (DtS), and regrowth (REG). FDR (5%) based significance thresholds for 17,211 SNPs indicated by dashed lines.

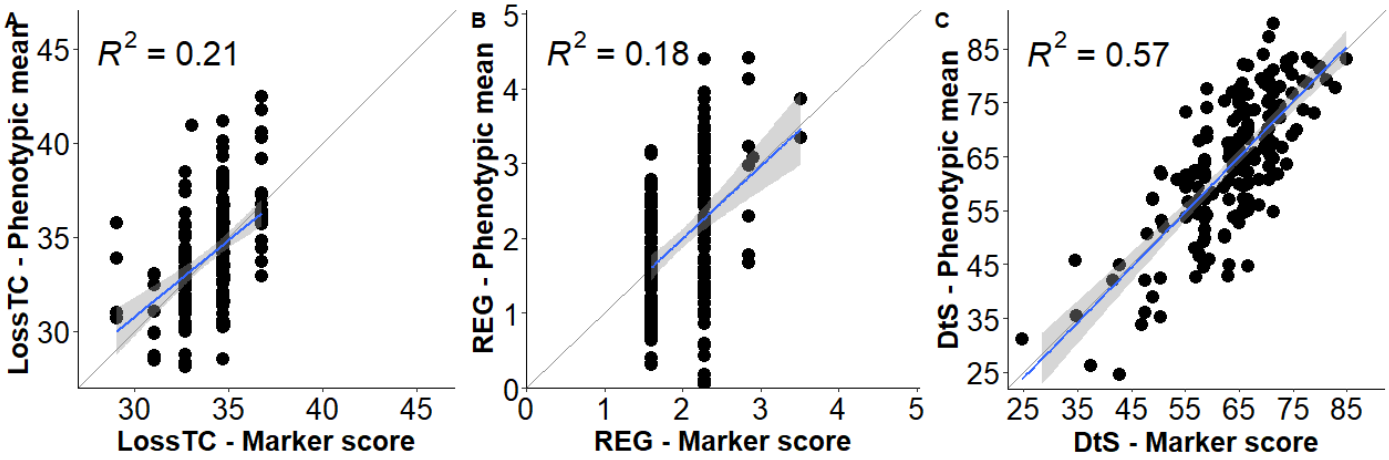

Figure 4 Correlation between calculated marker score per inbred line and phenotypic mean values per sub-trait (LossTC, loss of turgor and color [scale points]; DtS, disposition to survive [°]; REG, regrowth [g]). Marker score values were centered around the phenotypic mean values of the A-set lines (*n* = 183).

Further analysis of the local LD structure as well as haplotype and candidate gene analysis have to be conducted, to validate any pleiotropic effect of this putative QTL on not only LossTC and DtS but REG as well. The second region with a conspicuous concentration of putative QTL was on Chr 4. Within a distance of 103.49 Mb, the markers AX-416809725 and AX-416774047 for DtS and AX-181193117 for REG were found. However, none of the marker pairs was in an LD of r^2 >0.07. In addition, each marker pair distance (bp) was larger than the chromosome-wise LD decay region of Chr 4 (15,595,211.52 bp). According to these findings, these markers might not address the same putative QTL. Yet again, further investigations on the local LD pattern among the neighboring SNPs as well as haplotype and candidate gene analyses may resolve this uncertainty.

For each A-set line, a specific marker score was calculated based on its marker genotype. Correlating the marker scores to the true phenotypic mean values per sub-trait, revealed great discrepancies between the observed R^2 and the total sum of BLINK -based marker PVE, respectively. In the case of LossTC, the total sum of marker PVE (36.47%) was nearly twice as large as the R^2 (20.61%, marker score-based, Fig. 4A). The same holds true for REG with a total sum of 32.33% PVE and R^2 = 17.87% (Fig. 4B). These findings indicate that a large proportion of the sub-trait phenotypic variance was not captured by the identified putative QTL. However, LossTC even though showing a high h^2 (81.18%) is a complex highly quantitative sub-trait combining multiple aspects of hardening and dehardening response, such as proline accumulation and changes in fatty acid composition (Ali *et al.*, 2016; Link *et al.*, 2010). Therefore, the power of detection of this GWAS might have been too low due to the limited number of utilized inbred lines. Hence, smaller effect QTL may remain unrevealed for LossTC. Reasons for the low h^2 REG have been discussed already. They potentially affected the QTL detection for this sub-trait.

In the case of DtS, in contrast, the total sum of marker PVE (44.92%) explained about 10% less of the phenotypic variance than calculated from marker score (R^2 = 56.88%, Fig. 4C). According to this finding, the identified putative QTL might explain about half of the phenotypic variance for this sub-trait. As it was stated earlier, marker 6 and marker 8 might address the same putative QTL on Chr 4. However, the marker score-based R^2 was larger than the total sum PVE from BLINK output. This suggests that marker 6 and marker 8 were each addressing a unique putative QTL for DtS. If only one putative QTL was addressed by both markers, the R^2 would have been smaller than the total sum PVE. DtS did not only have the highest h^2 (81.43%) but also the highest number of detected markers compared to LossTC and REG. However, the h^2 of this sub-trait might differ from h^2 in field situation due to line-specific influences of the applied, drastic above ground biomass reduction on the survival behavior of the inbred lines. This may have induced a bias into the sub-trait DtS. Accordingly, the number of DtS related QTL detected with this protocol here may stay restricted, even if a larger panel of inbred lines was used. It is still up to further research whether cutting the plants above the second node after frost test (to assess REG) reflects the field conditions for DtS as good as assessing DtS without cutting.

Due to the design of the 60K Vfaba_v2 Axiom SNP array, most SNPs were located within annotated *V. faba* genes (D. O'Sullivan, pers. commun.). Thus, to get a first glimpse on potential candidate genes, we selected the highest effect marker for LossTC and tracked down the respective gene of SNP origin. Marker 2 (AX-416762439) was found to be in a gene encoding a protein kinase superfamily protein in *Arabidopsis* (At1g01540). It is involved in cellular response to lipids, defense response, fatty acid metabolic processes, and lipid biosynthetic processes for instance, a function that does not rule out this gene as a candidate (Arbaoui & Link, 2008). In *M. truncatula*, this gene (MTR_8g028695) was located on chromosome 8 and is described as putative serine/threonine-kinase. However, no further information about gene function was available. The actual candidate gene search is currently underway. We focus on an LD-based approach to examine the local LD structure in close vicinity around each putative QTL. We aim for narrowing down the genomic region within which potential candidate genes might most likely be located.

Subsequent studies will be conducted to validate the presented GWAS results for late-frost tolerance and finalize the candidate gene search. The genetic material for validation (the V-set inbred lines) is partially closely related to the A-set (same origin as described) or is inbred material from genebank accessions and

winter faba bean cultivars, such as 'Striker', 'Karl', and 'Diva'. In addition, we aim for verification of the final results by correlating the experimental climate chamber phenotypic data to overwintering data from field trials which were and are conducted with the same genetic material.

Acknowledgements

The authors would like to thank D. Angra and D.M. O'Sullivan for DNA extraction and genotyping, and for pre-processing the SNP data. This research was conducted mainly within the project ProFaba. ProFaba was carried out under the ERA-NET Cofund SusCrop (Grant No. 771134), being part of the Joint Programming Initiative on Agriculture, Food Security and Climate Change (FACCE -JPI). This research was funded by the Bundesministerium für Bildung und Forschung (BMBF, Grant No.: 031B0805A). A.W. acknowledges a scholarship from the Karl Eigen and Dr.h.c. Dietrich Brauer Foundation (UFOP, Union zur Förderung von Oel- und Proteinpflanzen e.V.).

References

Ali MBM, Welna GC, Sallam A, Martsch R, Balko C, *et al*. (2016) Association analyses to genetically improve drought and freezing tolerance of faba bean (*Vicia faba* L.). Crop Sci 56:1036-1048. DOI: 10.2135/CROPSCI2015.08.0503

Arbaoui M, Link W (2008) Effect of hardening on frost tolerance and fatty acid composition of leaves and stems of a set of faba bean (*Vicia faba* L.) genotypes. Euphytica 162:211-219. DOI: 10.1007/s10681-007-9521-4

Arbaoui M, Balko C, Link W (2008) Study of faba bean (*Vicia faba* L.) winter-hardiness and development of screening methods. Field Crop Res 106:60-67. DOI: 10.1016/j.fcr.2007.10.015

Berardini TZ, Reiser L, Li D, Mezheritsky Y, Muller R, *et al*. (2015) The Arabidopsis Information resource: Making and mining the "gold standard" annotated reference plant genome. Genesis 53:474-485. DOI: 10.1002/dvg.22877

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: A practical and powerful approach to multiple testing. J R Stat Soc Ser B Methodol 57:289-300.

Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, *et al*. (2007) TASSEL: Software for association mapping of complex traits in diverse samples. Bioinformatics 23:2633-2635. DOI: 10.1093/ bioinformatics/btm308

Carrillo-Perdomo E, Magnin-Robert J-B, Raffiot B, Deulvot C, Floriot M, *et al*. (2022) A QTL approach in faba bean highlights the conservation of genetic control of frost tolerance among legume species. Front Plant Sci 13:970865. DOI: 10.3389/fpls.2022.970865

Castel T, Lecomte C, Richard Y, Lejeune-Hénaut I, Larmure A (2017) Frost stress evolution and winter pea ideotype in the context of climate warming at a regional scale. OCL 24:D106. DOI: 10.1051/ocl/2017002

Gasim SE (2003) Winter-hardy faba bean: Applied genetic research on the reproductive mode of the European gene pool. Ph.D. thesis, Georg-August-Universität, Cuvillier Verlag, Göttingen.

Gasim S, Abel S, Link W (2004) Extent, variation and breeding impact of natural cross-fertilization in German winter faba beans. Euphytica 136:193-200. DOI: 10.1023/B:EUPH.0000030672. 56206.f0

Herzog H (1989) Influence of pre-hardening duration and dehardening temperatures on varietal freezing resistance in faba beans (*Vicia faba* L.). Agronomie 9:55-61. DOI: 10.1051/agro: 19890106

Huang M, Liu X, Zhou Y, Summers RM, Zhang Z (2019) BLINK: A package for next level of genome wide association studies with both individuals and markers in the millions. GigaScience 8:giy154. DOI: 10.1093/gigascience/giy154

Jayakodi M, Golicz AA, Kreplak J, Fechete LI, Angra D, *et al*. (2023) The giant diploid faba genome unlocks variation in a global protein crop. Nature 615:652-659. DOI: 10.1038/s41586-023-05791-5

Khazaei H, O'Sullivan DM, Stoddard FL, Adhikari KN, Paull JG, *et* al. (2021) Recent advances in faba bean genetic and genomic tools for crop improvement. Legume Sci 3:e75. DOI: 10.1002/leg3.75

Link W, Balko C, Stoddard FL (2010) Winter hardiness in faba bean: Physiology and breeding. Field Crop Res 115:287-296. DOI: 10.1016/j.fcr.2008.08.004

Money D, Gardner K, Migicovsky Z, Schwaninger H, Zhong GY, *et al*. (2015) LinkImpute: Fast and accurate genotype imputation for nonmodel organisms. G3-Genes Genomes Genet 5:2383-2390. DOI: 10.1534/g3.115. 021667

O'Sullivan DM, Angra D, Harvie T, Tagkouli V, Warsame A (2019) A genetic toolbox for *Vicia faba* improvement. International conference on legume genetics and genomics, May 13-17, Dijon, France.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, *et al*. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81:559-575. DOI: 10.1086/519795

Roth F, Link W (2010) Selektion auf Frosttoleranz von Winterackerbohnen (*Vicia faba* L.): Methodenoptimierung und Ergebnisse. 60. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs 2009, pp 31-37.

Sallam A, Martsch R, Moursi Y (2015) Genetic variation in morpho-physiological traits associated with frost tolerance in faba bean (*Vicia faba* L.). Euphytica 205:395-408. DOI: 10.1007/s10681–015–1395–2

Sass O (2022) Ackerbohnen und Erbsen – ist die Züchtung aus den Startlöchern? UFOP Pflanzenprotein Symposium „Local Heroes", 23 Nov, Berlin. [https://www.ufop.de/index.php/download_file/ view/12001/2049/; accessed 31 Jan 2023]

Skovbjerg CK, Angra D, Robertson-Shersby-Harvie T, Kreplak J, Keeble-Gagnère G, *et al*. (2022) Genetic analysis of global faba bean diversity, agronomic traits and selection signatures. Theor Appl Genet 136:114. DOI: 10.1007/s00122-023-04360-8

Statistisches Bundesamt (2023) Destatis: Ackerland nach Hauptfruchtgruppen und Fruchtarten. [https://www.destatis.de/DE/Themen/Branchen-Unternehmen/Landwirtschaft-Forstwirtschaft-Fischerei/Feldfruechte-Gruenland/Tabellen/ackerland-hauptnutzungsarten-kulturarten.html; accessed 24 Jan 2023]

Utz F (2011) PLABSTAT 3Awin: A computer program for statistical analysis of plant breeding experiments. Institute of Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, Stuttgart.

Wang J, Zhang Z (2021) GAPIT Version 3: boosting power and accuracy for genomic association and prediction. Genom Proteom Bioinform 19:629-640. DOI: 10.1016/j.gpb.2021.08.005

Yates AD, Allen J, Amode RM, Azov AG, Barba M, *et al*. (2022) Ensembl Genomes 2022: an expanding genome resource for non-vertebrates. Nucleic Acids Res 50:996-1003. DOI: 10.1093/nar/gkab1007

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Improving baking quality of wheat varieties by higher protein use efficiency and stability

Friedrich LAIDIG[1], Alexandra HÜSKEN[2], Dirk RENTEL[3], Hans-Peter PIEPHO[1]

[1] University of Hohenheim, Institute of Crop Science, Biostatistics Unit, Fruwirthstrasse 23, 70599 Stuttgart, Germany

[2] Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Department of Safety Quality of Cereals, Schuetzenberg 12, 32756 Detmold, Germany

[3] Bundessortenamt, Osterfelddamm 60, 30627 Hannover, Germany

(✉) Friedrich.Laidig@uni-hohenheim.de

Abstract

The most important trait for baking quality of winter wheat is loaf volume (V). It is mostly determined by grain protein content (P) and quality. New varieties with a high potential of grain protein use efficiency (ProtUE) are very important for reducing the surplus use of nitrogen fertilizer in areas of strong nitrogen leaching. This is also an important goal of agricultural policies in the European Union. Additionally, ProtUE needs to be very stable across environments in the face of progressing climate change with more volatile growing conditions.

We evaluated a new approach to assess ProtUE and stability based on the V-P relationship instead of using only single traits. We consider ProtUE as V divided by the amount of P (V/P). Highest ProtUE will be obtained if the protein quality is high and P is kept to a minimum (V is measured as the baking volume in mL produced by 100 g flour). The study comprised 11775 baking tests from 355 varieties grown from 1988 to 2019 in 668 different environments in Germany. We introduced a static and a dynamic model to assess ProtUE and stability as potential criteria in variety registration. Stability analyses were done separately for each variety. The dataset available for each variety comprised data from a complete testing cycle (trial series S1, S2, S3) and 8 locations in each series, with some possible overlap of locations in S1, S2 and S3. The total number of observations available per variety was $3 \times 8 = 24$. The static model was based on the ratio of V/P. The static ProtUE for a given variety can be calculated with the overall mean across all 24 observations, denoted by m_S. It represents the volume which can be achieved per 1% P on the average. The static stability measures the deviation of observations from year-wise means by the standard deviation s_S. The dynamic model represents the regression of V on P, where the regression coefficient is denoted as the dynamic ProtUE b_D. It indicates the increase of V per 1% of P. The standard deviation of the differences of observations from the regression line is the dynamic stability s_D. In Fig. 1, examples for varieties 'Genius' and 'Julius' are displayed, which demonstrated that 'Genius' has higher ProtUE and stability under both models.

If measures of ProtUE and stability are used as reliable characteristics for registration, they should have a high predictive power, *i.e.*, measures should be repeatable. We applied the heritability coefficient h^2 based on variance components of ProtUE and stability measurements m_S, s_S, b_D and s_D to evaluate their repeatability. Moreover, selection of varieties for static and dynamic ProtUE and stability should not counter-act the selection for high V and P (*i.e.*, the selection of varieties with high ProtUE and stability does not risk retention of varieties with low V). We therefore derived genotypic correlation coefficients for the association among cycle means of V, P and stability measures.

In general, heritability of ProtUE was higher than for stability. The highest heritability showed the static ProtUE m_S (h^2=92%), while the dynamic b_D was less than half of the static one (h^2=43%). The static stability s_S (h^2=51%) was considerably higher than the dynamic stability s_D (h^2=32%). We found no counter-action for the selection of high ProtUE and stability for both models. In particular, the correlation of the static ProtUE m_S with V and P was $r = 0.81$ and $r = 0.22$, and of the dynamic ProtUE b_D with V and P was $r = 0.49$ and $r = 0.40$, respectively. From these results we can conclude that high ProtUE is moderately to very strong associated with P and V. Static stability s_S was negatively correlated with V and P ($r = -0.24$ and $r = -0.28$), and dynamic stability s_D was uncorrelated with V ($r = -0.05$) and P ($r = 0.04$). We should notice that negative correlation of V and P with stability measures means that a higher stability is associated with higher values for V and P.

Our results showed that static ProtUE is highly repeatable and stability is moderately repeatable. They should be considered as additional efficient quality-related descriptors in breeding and testing of new winter wheat varieties with high potential for ProtUE and stability.

Keywords

Genotypic correlation · genotype by environment interaction · heritability · loaf volume · stability · *Triticum*

Laidig F, Hüsken A, Rentel D, Piepho HP (2023) Improving baking of wheat varieties by higher protein use efficiency and stability. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 37-38. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

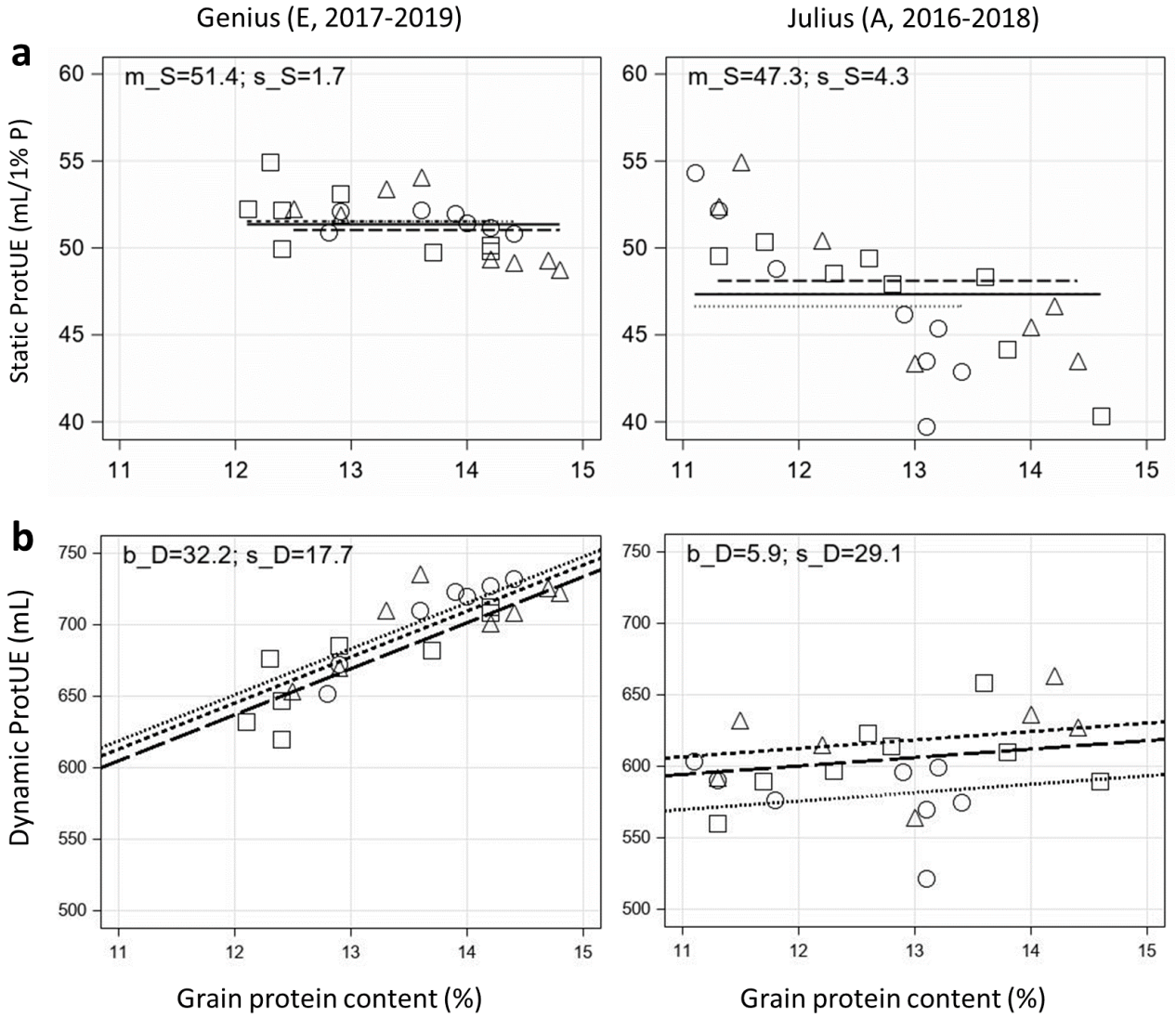

Figure 1 Examples demonstrating protein use efficiency (ProtUE) and stability for varieties 'Genius' and 'Julius': **a** static ProtUE and static stability; **b** dynamic ProtUE and dynamic stability. Circles, triangles and squares represent observations from first (S1), second (S2) and third (S3) test year, respectively. Mean and regression lines are plotted as dotted, short and medium dashed lines for S1, S2 and S3, respectively. For **a** the static ProtUE m_S is plotted as solid line. The estimated parameters are given as insets in the respective subplot: *m_S*, static ProtUE (m_S); *s_S*, static stability (s_S); *b_D*, dynamic ProtUE (b_D); *s_D*, dynamic stability (s_D).

References and further reading

Hüsken A (2020) Qualitätsweizenanbau in Deutschland: Die Praxis braucht einen neuen Stabilitäts- und Effizienzmaßstab. Getreide, Mehl und Brot 3:98-101.

Laidig F, Hüsken A, Rentel D, Piepho HP (2022) Protein use efficiency and stability of baking quality in winter wheat based on the relation of loaf volume and grain protein content. Theor Appl Genet 135:1331-1343. DOI: 10.1007/s00122-022-04034-x

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Effects of the high protein gene *Gpc-B1* on quality traits of organic wheat

Leon Michael KAMPL[1], Lucie DOSTALÍKOVÁ[2], Matilda CIUCA[3], Daniel CRISTINA[3], Marianna MAYER[4], Heinrich GRAUSGRUBER[1]

[1] Institute of Plant Breeding, Department of Crop Sciences, University of Natural Resources and Life Sciences, Konrad-Lorenz-Str. 24, 3430 Tulln an der Donau, Austria

[2] Department of Crop Sciences and Agroforestry, Faculty of Tropical AgriSciences, Czech University of Life Sciences, Prague, Czech Republic

[3] Laboratory of Molecular Genetics, National Agricultural Research and Development Institute Fundulea, Bucharest, Romania

[4] Cereal Breeding Department, Agricultural Institute, Centre for Agricultural Research, Martonvásár, Hungary

(✉) heinrich.grausgruber@boku.ac.at

Abstract

Grain protein content (GPC) of wheat is related to its end-use quality and is, therefore, a main quality trait for the international trade of common wheat (*Triticum aestivum*). In organic wheat production, GPC is generally lower than in conventional production due to lower nitrogen availability (Mäder *et al*., 2007; Bilsborrow *et al*., 2013; Mayer *et al*., 2015). Thus, thresholds for GPC of organic wheat are usually lower compared to conventional wheat (AMA, 2022). Various genes and QTL associated with a higher GPC were identified in wheat and molecular markers were developed (Distelfeld *et al*., 2006; Cormier *et al*., 2015; Rasheed *et al*., 2016; Jiang *et al*., 2021; Dixon *et al*., 2022). Of these genes, the functional *Gpc-B1* allele from wild emmer (*T. dicoccoides*), also known as *NAM-B1* (*NO APICAL MERISTEM-B1*), is the most widely exploited in wheat breeding (Tabbita *et al*., 2017).

We used *Gpc-B1* to evaluate its effect on ensuring high GPC and baking quality in organic wheat. The functional Gpc-B1 allele was transferred from hard-red spring wheat 'Glupro' (ND, USA) into winter wheats 'Spontan' (Germany) and 'Mv Kolompos' (Hungary). The allelic status of *Gpc-B1* was verified in 500 $F_{3:4}$ plants of each cross by the *Xuhw83* marker (Distelfeld *et al*., 2006) and/or the KASP marker GCP-DUP (Rasheed *et al*., 2016). Plants being homozygous for the functional and non-functional *Gpc-B1* alleles were bulked within each cross for seed multiplication in 2022 in Raasdorf, Austria. Seed multiplication was carried out under organic management with alfalfa as a pre-crop. Seeds of $F_{3:6}$ bulks were distributed to project partners in fall 2022 for multi-environment trials. Remaining seeds were used for preliminary quality analyses.

Grain protein content was determined by near-infrared spectoscopy using a NIRS™ DS2500 L machine (Foss GmbH, Hamburg). Dough mixing properties were evaluated with a micro-doughLAB (Perten Instruments, Hamburg). Dough extensibility was determined by a TA.XTplus texture analyzer equipped with the Kieffer dough and gluten extensibility rig (A/KIE) (Stable Micro Systems, Godalming). Dough samples (10 g) for the extensibility tests were prepared in a Promylograph T3 machine (M. Egger, St. Blasen) with manually adding the amount of water as determined by the micro-doughLAB. Mixing was done until peak resistance. Settings and analysis of extensibility curves were done by Exponent software (Stable Micro Systems) according to Grausgruber *et al*. (2002). Statistical analyses were carried out with SAS 9.4 software (SAS Institute, Cary, NC).

GPC was increased by ≈1% by *Gpc-B1* in both crosses, however, differences between the two bulks were not statistically significant (Table 1). Mixing properties showed increased times for dough development, mixing tolerance index and peak energy for *Gpc-B1* in both backgrounds, however, only in BTX501 (Spontan/Glupro) these differences were statistically significant. Dough softening was decreased in BTX501, but increased in BTX502 (Mv Kolompos/Glupro). Contradictory results in the two genetic backgrounds were also observed for the micro-extensograph method, *i.e*. slightly increased dough resistance but reduced extensibility in BTX501, whereas in BTX502 peak resistance was reduced and extensibility significantly increased.

The preliminary results showed that *Gpc-B1* efficiently increased GPC in both genetic backgrounds, however, effects were not coherent with respect to dough mixing and dough extensibility properties. The two recipient varieties used in this study are different especially with respect to their maturity but also baking quality which may be correlated with the different responses to the *Gpc-B1* introgression. Significant interactions of *Gpc-B1* with genotype and environment were reported (for review see *e.g*. Tabbita *et al*., 2017). *Gpc-B1* was shown to cause a more rapid onset of senescence in many genetic backgrounds and, thus, reduce grain weight (Uauy *et al*., 2006; Carter *et al*., 2012). In the previous study, thousand grain weight was reduced significantly by *Gpc-B1* in both crosses, *i.e*. by 5.3 g for BTX501 (45 vs. 39.7 g) and by 2 g for BTX502 (46.7 vs. 44.7 g). Within the ECOBREED project, multi-environment trials are currently ongoing which will help to unravel the interaction of *Gpc-B1* with the particular genotypes and environments with respect to agronomic and end-use quality related traits.

Kampl L, Dostalíková L, Ciuca M, Cristina D, Mayer M, *et al*. (2023) Effects of the high protein content gene *Gpc-B1* on quality traits of organic wheat. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 39-40. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

Table 1 Comparison of quality traits between bulks without (N) and with (*Gpc-B1*) the functional *Gpc-B1* allele in two different common wheat crosses. Means with different letters within a cross are significantly different at $\alpha = 0.05$.

	BTX501 (Spontan/Glupro)		BTX502 (Mv Kolompos/Glupro)	
Trait	N	*Gpc-B1*	N	*Gpc-B1*
Protein content (%)	13.9 [a]	15.0 [a]	15.9 [a]	16.9 [a]
µ-doughLAB				
Dough development time (min)	6.6 [b]	12.3 [a]	6.2 [a]	7.3 [a]
Dough softening (FU)	65.0 [a]	65.0 [a]	62.5 [a]	82.5 [a]
Mixing tolerance index	71.7 [b]	74.4 [a]	68.9 [a]	70.4 [a]
Peak energy (Wh kg^{-1})	8.8 [b]	16.6 [a]	8.4 [a]	9.4 [a]
Quality number	64.0 [a]	67.5 [a]	63.7 [a]	58.4 [a]
Kieffer dough extensibility rig				
Peak resistance (g)	23.1 [a]	23.9 [a]	20.4 [a]	14.2 [b]
Extensibility to peak (mm)	74.9 [a]	61.1 [b]	73.9 [b]	90.3 [a]
Dough energy to peak (g mm)	1322 [a]	1164 [a]	1131 [a]	1036 [b]
Kurzextensogrammzahl	104 [a]	89 [a]	94 [a]	97 [a]
Relationszahl	32.4 [a]	25.8 [a]	37.4 [b]	63.5 [a]

Keywords

Dough rheology · end-use quality · grain protein content · organic farming · *Triticum aestivum*

Acknowledgements

L.D. acknowledges support by ERASMUS+ for a traineeship at BOKU Vienna. This work received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 771367 (ECOBREED).

References and further reading

AMA (2022) Bio-Erzeugerpreise endgültig, Österreich, Getreide-wirtschaftsjahr 2021/2020. Daten & Fakten der AgrarMarkt Austria (AMA) für den Bereich Getreide und Ölsaaten, Wien.

Bilsborrow P, Cooper J, Tétard-Jones C, Średnicka-Tober D, Barański M, *et al*. (2013) The effect of organic and conventional management on the yield and quality of wheat grown in a long-term field trial. Eur J Agron 51:71-80. DOI: 10.1016/j.eja.2013.06.003

Carter AH, Santra DK, Kidwell KK (2012) Assessment of the effects of the *Gpc-B1* allele on senescence rate, grain protein concentration and mineral content in hard red spring wheat (*Triticum aestivum* L.) from the Pacific Northwest Region of the USA. Plant Breed 131:62-68. DOI: 10.1111/j.1439-0523.2011.01900.x

Cormier F, Throude M, Ravel C, Le Gouis J, Leveugle M, *et al*. (2015) Detection of *NAM-A1* natural variants in bread wheat reveals differences in haplotype distribution between a worldwide core collection and European elite germplasm. Agronomy 5:143-151. DOI: 10.3390/agronomy5020143

Distelfeld A, Uauy C, Fahima T, Dubcovsky J (2006) Physical map of the wheat high-grain protein content gene *Gpc-B1* and development of a high-throughput molecular marker. New Phytol 169:753-763. DOI: 10.1111/j.1469-8137.2005.01627.x

Dixon LE, Pasquariello M, Badgami R, Levin KA, Poschet G, *et al*. (2022) MicroRNA-resistant alleles of *HOMEOBOX DOMAIN-2* modify inflorescence branching and increase grain protein content of wheat. Sci Adv 8:eabn5907. DOI: 10.1126/sciadv.abn5907

Grausgruber H, Schöggl G, Ruckenbauer P (2002) Investigations on the validity of the micro-extensigraph method to measure rheological properties of wheat doughs. Eur Food Res Technol 214:79-82. DOI: 10.1007/s002170100399

Jiang P, Zhang P, Wu L, He Y, Li C, *et al*. (2021) Linkage and association mapping and Kompetitive Allele-Specific PCR marker development for improving grain protein content in wheat. Theor Appl Genet 134:3563-3575. DOI: 10.1007/s00122-021-03913-z

Mäder P, Hahn D, Dubois D, Gunst L, Alföldi T, *et al*. (2007) Wheat quality in organic and conventional farming: results of a 21 year field experiment. J Sci Food Agric 87:1826-1835. DOI: 10.1002/jsfa.2866

Mayer J, Gunst L, Mäder P, Samson M-F, Carcea M, *et al*. (2015) Productivity, quality and sustainability of winter wheat under long-term conventional and organic management in Switzerland. Eur J Agron 65:27-39. DOI: 10.1016/j.eja.2015.01.002

Rasheed A, Wen W, Gao F, Zhai S, Jin H, *et al*. (2016) Development and validation of KASP assays for genes underpinning key economic traits in bread wheat. Theor Appl Genet 129:1843-1860. DOI: 10.1007/s00122-016-2743-x

Tabbita F, Pearce S, Barneix AJ (2017) Breeding for increased grain protein and micronutrient content in wheat: Ten years of the *GPC-B1* gene. J Cereal Sci 73:183-191. DOI: 10.1016/j.jcs.2017.01.003

Uauy C, Distelfeld A, Fahima T, Blechl A, Dubcovsky J (2006) A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. Science 314:1298-1301. DOI: 10.1126/science.1133649

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Baking quality of organic heterogeneous material of wheat

Caroline HEIDEN[1], Stefano D'AMICO[2], Elisabeth REITER[2], Clemens FLAMM[2], Heinrich GRAUSGRUBER[1]

[1] Institute of Plant Breeding, Department of Crop Sciences, University of Natural Resources and Life Sciences, Konrad-Lorenz-Str. 24, 3430 Tulln an der Donau, Austria

[2] Austrian Agency for Health and Food Safety (AGES), Spargelfeldstr. 191, 1220 Vienna, Austria

(✉) stefano.d-amico@ages.at

Abstract

The new regulation on organic production (EU) 2018/848 entered into force on January 1, 2022, and facilitates a simple notification process of organic heterogeneous material (OHM) and regulates the marketing of seeds as laid down in regulation (EU) 1189/2021. OHM is not a seed mixture that is rebuild annually based on varieties (*i.e*. variety mixture) but characterised by a high level of phenotypic and genetic diversity within a single botanic taxon, and its dynamic nature to evolve and adapt to various biotic and abiotic stresses due to repeated natural and human selection and therefore is expected to change over time (EC, 2021). While genetic heterogeneity may be an advantage with respect to stress tolerance and yield stability, it may be not questionable with respect to end-use quality.

To evaluate the end-use quality of currently available OHM, a field trial was established at two sites in Austria (Großnondorf and Zinsenhof) and one site in Switzerland (Nyon) with six winter wheat varieties originating from Austria ('Aurelius'), Germany ('Bernstein') and Switzerland ('Baretta', 'Montalbano', 'Pizza', 'Wiwa') and three OHMs ('Brandex Population', 'Mv Elit CCP', 'Solibam Floriddia'). Additionally, three variety mixtures (Aurelius:Bernstein, Baretta:Montalbano, Wiwa:Pizza; all 1:1) were included. The three OHMs have different history of development and genetic origin: 'Brandex' was developed from a mixture of 10 bulks of multi-parental crosses and selected for common bunt resistance after artificial inoculation; 'Mv Elit CCP' was developed from a half-diallel including six Hungarian varieties of similar phenotype with respect to plant height, maturity and awnedness; 'Solibam Floriddia' was developed from early-generations of multi-parental crosses of ICARDA's common wheat programme including in total about 200 parental lines. The field trials were carried out under both conventional and organic management in the 2020/2021 vegetation period. After harvest, samples bulked across replicates were analysed for ash content (ICC Standard Method 104/1), crude protein content (ICC 159), Zeleny sedimentation volume (ICC 116/1), Hagberg falling number (ICC 107/1), wet gluten content and gluten index (ICC 155). Dough rheological properties were determined by the farinograph (ICC 115/1) and the extensograph (ICC 114/1). Baking tests were carried out according to the Austrian *Kaisersemmel* (rolls with a rosette shape) baking test; volume measurements were done by a VolScan VSP600 machine (Stable Micro Systems, Godalming, UK). The Swiss samples were finally removed from quality analyses as the grains were heavily damaged by *Sitotroga cerealella* larval feeding. Hence, we report here only results from the Austrian trials. Data were analysed by Genstat 22nd Ed. Software (VSNi, Hemel Hempstead, UK) fitting a linear mixed model ANOVA with gentoypes and management system as fixed effects and test sites as random effects.

In general, grain yield was higher for the conventional trials (89.1 dt ha^{-1}) compared to the organic trials (86.7 dt ha^{-1}), however, the gap between the two management systems was significantly smaller than the usually realized 20-30% (de Ponti *et al*., 2012). We observed a cross-over interaction between genotypes and management with 'Aurelius' and the mixture Baretta:Montalbano (Bar:Mon) performing superior under organic management (Table 1). Higher test weights were observed for the organic trials where each variety reached the Austrian base value of 78 kg hL^{-1} for organic wheat, whereas the base value for conventional wheat of 80 kg hL^{-1} was missed by 'Baretta', the Bar:Mon mixture and all three OHMs. For protein content the base value of 14% for conventional *Qualitätsweizen* was not reached by 'Aurelius', 'Bernstein' and their mixture, whereas all genotypes significantly outreached also the respective base value of 13% for organic *Premiumweizen*. The lower protein contents under conventional management were caused by significantly lower values at Zinsenhof compared to Großnondorf, whereas under organic management there was no difference between the two test sites (Fig. 1). Correlation between grain yield and protein content was significantly negative (r = -0.51, p < 0.001). Similar to the study of Baresel *et al*. (2022) higher protein contents but lower grain yields were observed for varieties derived from an organic breeding programme and for OHMs. Limits for Hagberg falling number were reached by each genotype with the exception of 'Solibam Floriddia', with a mean of 219 s for the organic samples being slightly below the base value of 220 s for *Mahlweizen*. Dough rheological parameters and baking volumes were generally higher for the organic samples, however, only for baking volume the difference between the management systems was statistically

Heiden C, D'Amico S, Reiter E, Flamm C, Grausgruber H (2023) Baking quality of organic heterogeneous material of wheat. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 41-44. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

Table 1 Best linear unbiased estimators (BLUEs) for pure line varieties, variety mixtures and OHM (populations) for the Austrian test sites (s.e.d. = standard error of differences for the respective fixed effects; significance levels for the fixed effects: n.s. = not significant at $p = 0.1$, † $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

	Grain yield (dt/ha)		Test weight (kg/hL)		Protein content (%)	
Genotype	con	org	con	org	con	org
Aurelius	97.6	101.5	82.1	83.8	13.4	14.2
Bernstein	98.1	87.5	81.0	82.3	13.1	14.8
Aur:Ber	96.6	94.8	81.4	83.0	13.5	14.9
Baretta	88.9	85.4	77.1	78.5	14.3	16.0
Montalbano	92.6	90.3	80.1	82.2	14.1	16.2
Bar:Mon	91.9	92.7	78.6	79.7	14.3	15.9
Pizza	86.7	79.9	82.2	83.0	14.6	16.4
Wiwa	84.4	78.7	81.2	82.1	14.6	16.4
Piz:Wiw	84.6	80.1	82.0	83.0	14.6	16.6
Brandex	91.4	87.6	79.4	81.2	14.4	15.8
Mv Elit CCP	78.7	91.7	77.9	79.9	14.7	15.6
Solibam	77.5	70.3	78.5	80.8	15.6	17.7
Mean	89.1	86.7	80.1	81.6	14.3	15.9
s.e.d. $_{GEN}$	2.52 ***		1.09 ***		1.19 $^{n.s.}$	
s.e.d. $_{MAN}$	1.03 *		0.45 **		0.49 **	
s.e.d. $_{GEN\times MAN}$	3.56 *		1.54 $^{n.s.}$		1.68 $^{n.s.}$	

	Farinogram quality number (FQN)		Extensogram energy (cm²)		Baking volume (mL/100 g)	
Genotype	con	org	con	org	con	org
Aurelius	80	113	164	174	448	486
Bernstein	82	113	130	162	475	537
Aur:Ber	128	116	142	172	463	512
Baretta	97	122	140	150	524	568
Montalbano	107	116	126	141	418	453
Bar:Mon	103	114	150	151	468	510
Pizza	78	109	159	183	496	572
Wiwa	72	93	129	127	511	556
Piz:Wiw	80	96	143	154	499	553
Brandex	61	85	99	114	442	514
Mv Elit CCP	68	70	71	66	486	483
Solibam	34	31	49	43	422	438
Mean	82	98	125	136	471	515
s.e.d. $_{GEN}$	1.09 $^{n.s.}$		15.4 ***		37.9 †	
s.e.d. $_{MAN}$	10.9 $^{n.s.}$		6.3 †		15.5 **	
s.e.d. $_{GEN\times MAN}$	37.9 $^{n.s.}$		21.8 $^{n.s.}$		53.6 $^{n.s.}$	

significant ($p = 0.009$). Despite some individual cross-over interaction, the overall genotype by management interaction was statistically not significant for the quality traits.

Although the OHMs delivered weak dough properties as indicated by FQN and extensogram energy, baking volume and quality were acceptable for 'Brandex Population' and 'Mv Elit CCP' which would, thus, be classified as *Mahlweizen.* On the contrary, 'Solibam Floriddia' can be classified only as feed; despite it exhibited the highest protein content it has obviously the poorest gluten quality which is also revealed by a poor dough processing quality, visible in an unclear formation of rosette of the rolls.

However, it must be considered that all tested OHMs were originally not developed for the test environments and that the presented results are hitherto derived only from two test sites and one experimental year. Nonetheless, it is obvious that the development of wheat populations with stable and high grain yields and excellent baking quality is challenging and needs a careful selection of parents well adapted to the respective target environments. Hitherto, many studies carried out on wheat populations considered only grain yield, yield stability, and quality traits relevant for marketing (*i.e*. protein content and falling number) but no further quality traits such as dough rheology and baking volume (Döring *et al*., 2015; Brumlop *et al*., 2017; Baresel *et al*., 2022). Therefore, more research is needed to study and develop wheat OHM also more with respect to gluten quality and processing quality.

Keywords

Dough rheology · end-use quality · genetic diversity · organic farming · population · *Triticum aestivum*

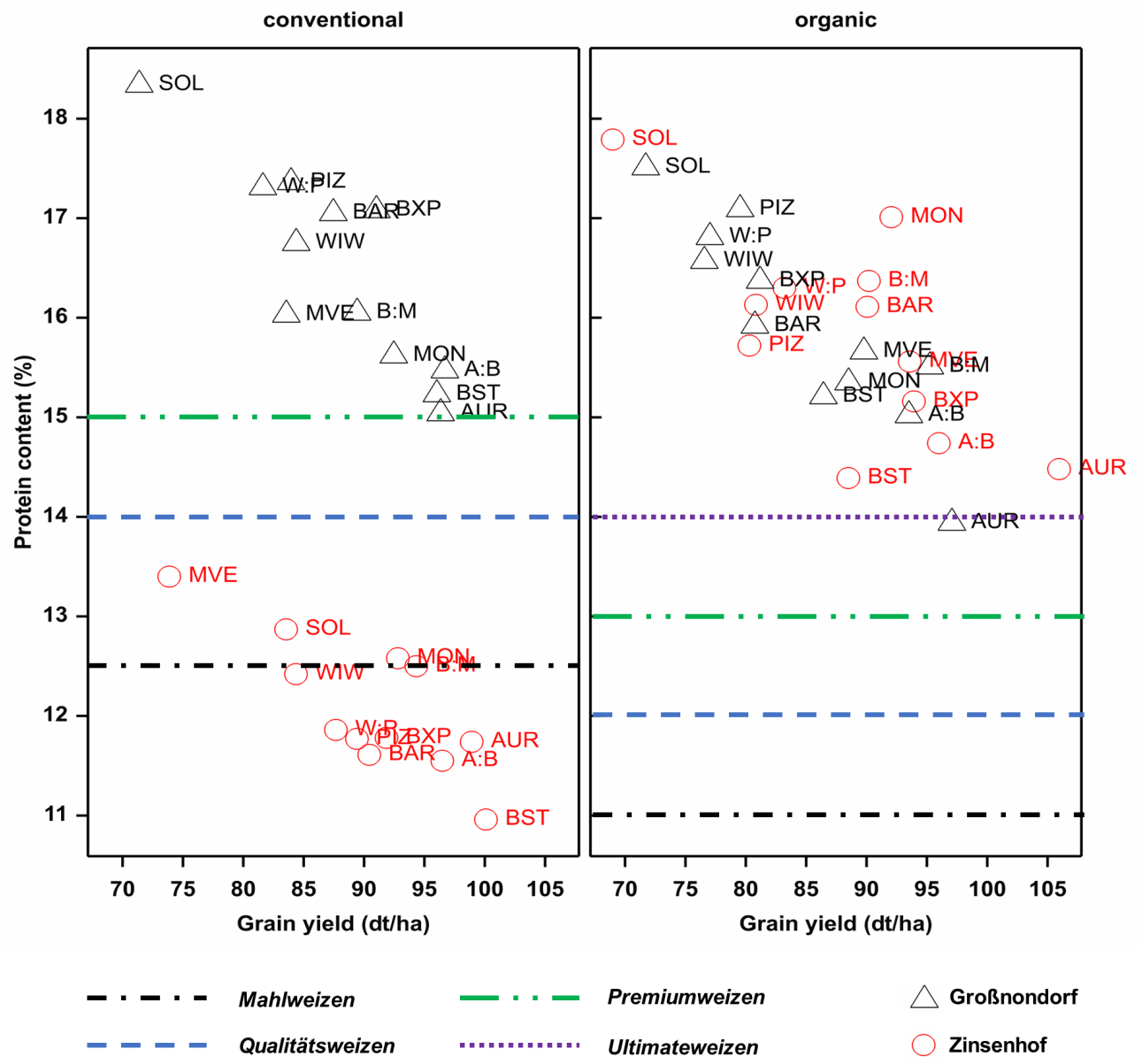

Figure 1 Grain yield and protein content of pure line varieties, variety mixtures and wheat populations (OHMs) under organic and conventional management in Großnondorf and Zinsenhof, Austria, 2021. Threshold values for protein content for organic and conventional wheat according to Agrarmarkt Austria (AMA) are indicated by different dotted lines. Genotype codes: AUR, Aurelius; BST, Bernstein; BAR, Baretta; MON, Montalbano; PIZ, Pizza; WIW, Wiwa; BXP, Brandex Population; MVE, Mv Elit CCP; SOL, Solibam Floriddia; A:B, Aurelius:Bernstein 1:1 mixture; B:M, Baretta:Montalbano 1:1 mixture; P:W, Pizza:Wiwa 1:1 mixture.

Acknowledgements

This work received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 817970 (INVITE). C.H. acknowledges scholarships by the Austrian Research Promotion Agency (FFG; FEMtech programme) and ICC Austria.

References and further reading

Baresel JP, Bülow L, Finckh MR, Frese L, Knapp S, *et al*. (2022) Performance and evolutionary adaptation of heterogeneous wheat populations. Euphytica 218: 137. DOI: 10.1007/s10681-022-03072-2

Brumlop S, Pfeiffer T, Finckh MR (2017) Evolutionary effects on morphology and agronomic performance of three winter wheat composite cross populations maintained for six years under organic and conventional conditions. Organic Farming 3: 34-50. DOI: 10.12924/of2017.03010034

Brumlop S, Weedon O, Link W, Finckh MR (2019) Effective population size (N_e) of organically and conventionally grown composite cross winter wheat populations depending on generation. Eur J Agron 109: 125922. DOI: 10.1016/j.eja.2019.125922

Ceccarelli S, Grando S (2020) Evolutionary plant breeding as a response to the complexity of climate change. iScience 23: 101815. DOI: 10.1016/j.isci.2020.101815

de Ponti T, Rijk B, van Ittersum MK (2012) The crop yield gap between organic and conventional agriculture. Agric Syst 108:1-9. DOI: 10.1016/j.agsy.2011.12.004

Döring T, Annicchiarico P, Clarke S, Haigh Z, Jones HE, *et al*. (2017) Comparative analysis of performance and stability among composite cross populations, variety mixtures and pure lines of winter wheat in organic and conventional cropping systems. Field Crops Res 183: 235-245. DOI: 10.1016/j.fcr.2015.08.009

EC (2021) Commission delegated Regulation (EU) 2021/1189 of 7 May 2021 supplementing Regulation (EU) 2018/848 of the European Parliament and of the Council as regards the production and marketing of plant reproductive material of organic heterogeneous material of particular genera or species. Official Journal of the European Union L258/18. European Commission (EC), Brussels.

Harlan HV, Martini ML (1929) A composite hybrid mixture. J Am Soc Agron 21: 487-490.
DOI: 10.2134/agronj1929.00021962002100040014x

Harlan HV, Martini ML (1938) The effect of natural selection in a mixture of barley varieties. J Agric Res 57: 189-199. https://handle.nal.usda.gov/10113/IND43969091

Knapp S, Döring TF, Jones HE, Snape J, Wingen LU, *et al*. (2020) Natural selection towards wild-type in composite cross populations of winter wheat. Front Plant Sci 10: 1757. DOI: 10.3389/fpls.2019.01757

Raggi L, Ciancaleoni S, Torricelli R, Terzi V, Ceccarelli S, *et al*. (2017) Evolutionary breeding for sustainable agriculture: selection and multi-environment evaluation of barley populations and lines
Field Crops Res 204: 76-88. DOI: 10.1016/j.fcr.2017.01.011

Suneson CA (1956) An evolutionary plant breeding method. Agron J 48: 188-191. DOI: 10.2134/agronj1956.00021962004800040012x

Suneson CA, Stevens H (1953) Studies with bulked hybrid populations of barley. USDA Tech BulL 1067. DOI: 10.22004/ag.econ.156533

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Characterization of wheat flour proteome and identification of genomic regions associated with gluten and allergenic proteins

Khaoula EL HASSOUNI[1], Muhammad AFZAL[1], Malte SIELAFF[2], Stefan TENZER[2], C. Friedrich H. LONGIN[1]

[1] University of Hohenheim, State Plant Breeding Institute, Fruwirthstr. 21, 70599 Stuttgart, Germany

[2] University Medical Center of the Johannes Gutenberg University Mainz, Institute for Immunology, Langenbeckstr. 1, 55131 Mainz, Germany

(✉) khaoula.elhassouni@uni-hohenheim.de

Abstract

Wheat is an important staple crop and its proteins contribute to human and animal nutrition, are important for its end-use quality, but can also cause adverse human reactions in a sizeable population. We performed a genome wide association study (GWAS) on 114 proteins, which were environmentally stable expressed across 148 wheat cultivars with a heritability >0.6. For 54 proteins, we were able to detect quantitative trait loci (QTL) surpassing the Bonferroni-corrected significance threshold and explaining 17.3–84.5% of the genotypic variance. Proteins of the same family often clustered at a very close or the potential homeolog chromosomal position. Major QTL were found for four well-known glutenin and gliadin subunits, and the QTL segregation pattern at the protein encoding the high molecular weight glutenin subunit *Dx5* could be confirmed by SDS gel-electrophoreses. For nine potential allergenic proteins, large QTL could be identified, and their measured allele frequencies opens the possibility to select for low protein abundancy by markers as long as their relevance for human health is finally proven. One potential allergen (*i.e.* prot288, protease inhibitor) was introduced consequently across the decades of wheat breeding starting in the 1980s, which might be linked to the cluster of rust resistance genes *Sr38-Yr17-Lr34* introgressed on chromosome 2AS from *Triticum ventricosum* (Fig. 1). The reported sequence information for the 54 major QTL can be used to design efficient markers for future wheat breeding.

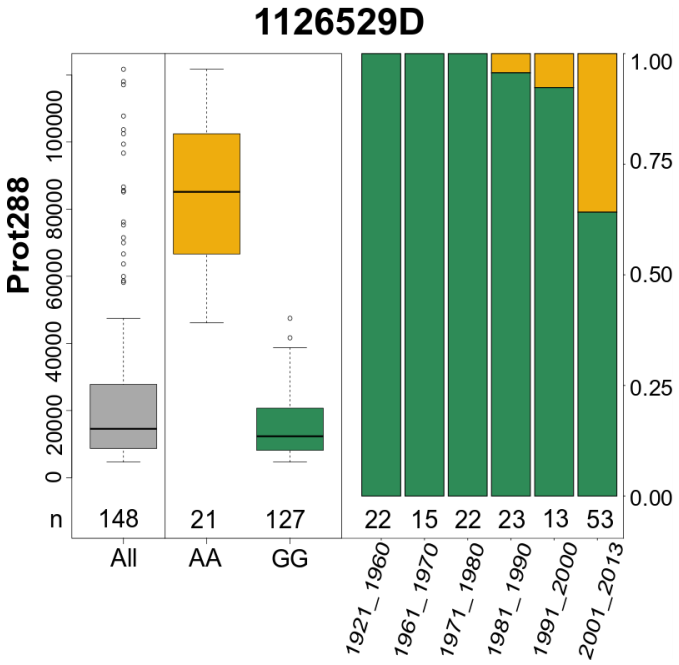

Figure 1 The effect of the major QTL for non-gluten allergenic protein prot288 and its allele frequencies according to the cultivar's year of release (numbers below boxes represent the number of cultivars in the respective group). The protein-increasing allele is colored in orange, the protein-decreasing allele is colored in green.

Keywords

Alien gene introgression · allergen · breeding · GWAS · non-gluten wheat sensitivity · quality · QTL · *Triticum aestivum*

Acknowledgements

The project was supported by Deutsche Forschungsgemeinschaft (DFG) to C.F.H.L. (LO 1816-4/1), and S.T. (TE599-3/1). K.E.H. was supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support program (BETTERWHEAT–FKZ: 2818405A18, 2818405B18).

El Hassouni K, Afzal M, Sielaff M, Tenzer S, Longin CFH (2023) Characterization of wheat flour proteome and identification of genomic regions associated with gluten and allergenic proteins. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 45-46. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

References and further reading

Afzal M, Sielaff M, Curella V, Neerukonda M, El Hassouni K, *et al*. (2021) Characterization of 150 wheat cultivars by LC-MS-based label-free quantitative proteomics unravels possibilities to design wheat better for baking quality and human health. Plants 10:424. DOI: 10.3390/plants10030424

Afzal M, Sielaff M, Distler U, Schuppan D, Tenzer S, *et al*. (2023) Reference proteomes of five wheat species as starting point for future design of cultivars with lower allergenic potential. NPJ Sci Food 7:9. DOI: 10.1038/s41538-023-00188-0

El Hassouni K, Afzal M, Steige KA, Sielaff M, Curella V, *et al*. (2023) Multiomics based association mapping in wheat reveals genetic architecture of quality and allergenic related proteins. Int J Mol Sci 24:1485. DOI: 10.3390/ijms24021485

El Hassouni K, Sielaff M, Curella V, Neerukonda M, Leiser W, *et al*. (2021) Genetic architecture underlying the expression of eight α-amylase trypsin inhibitors. Theor Appl Genet 134:3427-3441. DOI: 10.1007/s00122-021-03906-y

Ishikawa G, Nakamura T (2007) A new co-dominant PCR-based marker to identify the high-molecular-weight glutenin subunit combination “5+10” of common wheat. Wheat Inf Serv 103:1-4.

Schuppan D, Pickert G, Ashfaq-Khan M, Zevallos V (2015) Non-celiac wheat sensitivity: Differential diagnosis, triggers and implications. Best Pract Res Clin Gastroenterol 29:469-476. DOI: 10.1016/j.bpg.2015.04.002

Seah S, Bariana H, Jahier J, Sivasithamparam K, Lagudah ES (2001) The introgressed segment carrying rust resistance genes *Yr17*, *Lr37* and *Sr38* in wheat can be assayed by a cloned disease resistance gene-like sequence. Theor Appl Genet 102:600-605. DOI: 10.1007/s001220051686

Shewry PR, Hey S (2015) The contribution of wheat to human diet and health. Food Energy Secur 4:178-202. DOI: 10.1002/fes3.64

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Exploration of the immunogenic properties of amylase-trypsin inhibitors in wheat

Lisa CALL[1], Andrea KRISAI[1], Heinrich GRAUSGRUBER[1]

[1] University of Natural Resources and Life Sciences, Vienna, Department of Crop Sciences, Institute of Plant Breeding, Konrad Lorenz Str. 24, 3430 Tulln an der Donau, Austria

(✉) lisa.call@boku.ac.at

Abstract

While wheat amylase-trypsin inhibitors (ATIs) have been shown to have allergenic properties, their involvement in celiac disease and other wheat-related diseases is still debated. Recent studies suggest that ATIs are not only the main triggers of baker's asthma, a respiratory form of wheat allergy, but are also involved in the pathogenesis of non-celiac wheat senstivity (Junker *et al*., 2012) and celiac disease, as immune responses to ATIs have been detected in serum samples from celiac patients (Huebener *et al*., 2015; Sánchez *et al*., 2018). Although ATIs have a potentially significant impact on human health, little detailed information on their immunoreactivity is available. One of the major challenges in studying ATIs and their involvement in wheat-related diseases is the lack of pure ATI preparations that can be used for testing. In this study, a procedure for the purification of ATIs is presented with a series of subsequent analytical methods to verify the final purity of the extract. In addition, heterologous expression of the major ATI isoforms (0.19, CM1, and CM3) was performed in *Pichia pastoris* to provide an alternative strategy for producing ATI preparations in the absence of other immunoreactive proteins.

The selective purification of natural ATIs (nATIs) from wheat flour (*Triticum aestivum* cv. 'Arnold') was based on their biochemical properties, and included selective extraction in salt solutions (phosphate-buffered saline) and chloroform/methanol (CM) mixtures, as well as size-exclusion chromatography and affinity purification using trypsin. Several analytical methods were applied to evaluate the purity of the final nATI extract, *i.e*. SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis), MALDI-TOF MS (matrix-assisted laser desorption ionization - time of flight mass spectrometry), LC-MS/MS (liquid chromatography tandem mass spectrometry), and immunoblotting. Alternatively, *P. pastoris* was used as an expression system for the production of selected ATI proteins (*i.e*. 0.19, CM1, CM3) according to Tundo *et al*. (2018). The recombinant ATIs (rATIs) were purified by ion-exchange and size-exclusion chromatography (Fig. 1). In addition to purity analysis, the rATIs were also analyzed for their *in vitro* functionality (α-amylase and trypsin inhibitory activity) and 3D structure by CD (circular dichroism) analysis.

While SDS-PAGE and MALDI-TOF analyses yielded promising results for the purity of the nATI extract, LC-MS/MS and immunoblotting revealed the presence of gliadins (major wheat allergens) and other minor allergenic proteins. In contrast, the high purity of the rATIs expressed in this study was demonstrated by LC-MS/MS. However, evaluation of the CD spectra suggested that the recombinant proteins were not folded correctly during expression in *P. pastoris*. Consequently, no α-amylase or trypsin inhibitory activity was detected for rATIs, indicating that the 3D structure of ATIs is essential for their functionality.

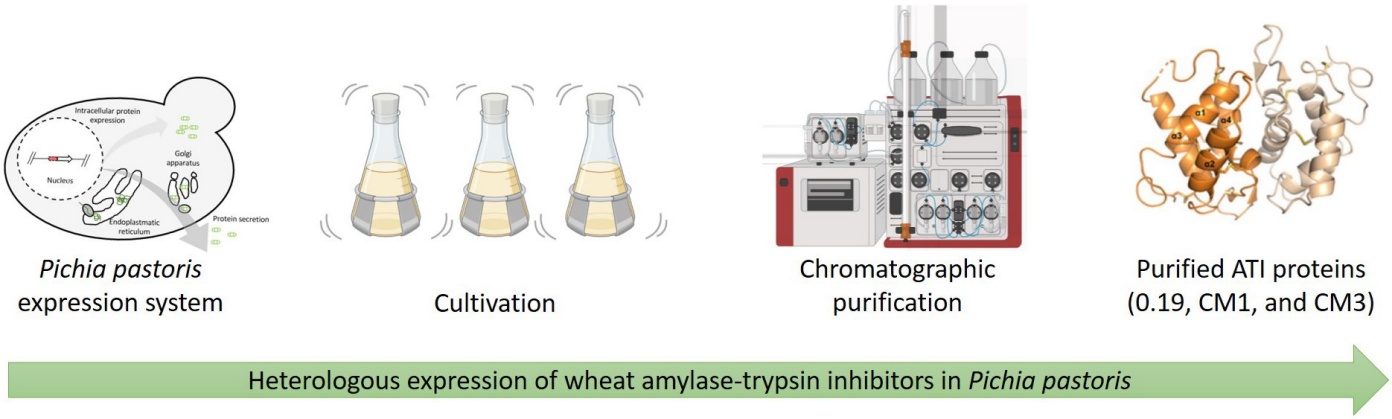

Figure 1 Schematic illustration of the expression of ATIs (isoforms 0.19, CM1, and CM3) in *Pichia pastoris*, including large-scale cultivation and chromatograhic purification (by ion-exchange and size-exclusion), with the aim of producing pure ATI preparations for biochemical and immunogenic characterization.

Call F, Krisai A, Grausgruber H (2023) Exploration of the immunogenic properties of amylase-trypsin inhibitors in wheat. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 47-49. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

As the involvement of wheat ATIs in celiac disease and non-celiac wheat sensitivity is still under discussion, the ability to produce specific ATI proteins with high purity and known composition is fundamental to elucidate their immunoreactive properties (*e.g.*, using the microarray chip technology established at the Department of Pathophysiology and Allergy Research at the Medical University of Vienna, Austria) and contribution to the adverse reactions observed *in vitro* and *in vivo*. This study demonstrated that even an elaborate procedure including multiple purification steps based on the biochemical properties of ATIs does not yield pure ATIs. Given this fact, the heterologous expression of recombinant ATI proteins is considered a promising alternative strategy. However, this method still needs to be optimized as target proteins lack disulfide bond formation, which is essential not only for their 3D structure but also for their functionality and probably their immunoreactivity.

Keywords

Allergy · heterologous expression · immunoreactive proteins · *Pichia pastoris* · *Triticum*

Acknowledgements

This research was funded by the Government of Lower Austria for work within the Danube Allergy Research Cluster (K3-T-74/001-2019). L.C. additionally acknowledges funding from the Institut française d'Autriche and the French Ministry of Europe and Foreign Affairs in the frame of a scientific research stay scholarship. The authors want to thank the project partners Birgit Linhart and Rudolf Valenta, Medical University of Vienna, for providing support, rabbit antisera for immunoblotting, and the CD instrument for secondary structure estimation.

References and further reading

Geisslitz S, Shewry P, Brouns F, America AHP, Caio GPI, *et al*. (2021) Wheat ATIs: Characteristics and role in human disease. Front Nutr 8:667370. DOI: 10.3389/fnut.2021.667370

Geisslitz S, Weegels P, Shewry P, Zevallos V, Masci S, *et al*. (2022) Wheat amylase/trypsin inhibitors (ATIs): occurrence, function and health aspects. Eur J Nutr 61:2873-2880. DOI: 10.1007/s00394-022-02841-y

Junker Y, Zeissig S, Kim SJ, Barisani D, Wieser H, *et al*. (2012) Wheat amylase trypsin inhibitors drive intestinal inflammation via activation of toll-like receptor 4. J Exp Med 209:2395-2408. DOI: 10.1084/jem.20102660

Huebener S, Tanaka CK, Uhde M, Zone JJ, Vensel WH, *et al*. (2015) Specific nongluten proteins of wheat are novel target antigens in celiac disease humoral response. J Proteome Res 14: 503-511. DOI: 10.1021/pr500809b

Sánchez D, Štěpánová Honzová S, Hospodková M, Hoffmanová I, Hábová V, *et al*. (2018) Occurrence of serum antibodies against wheat alpha-amylase inhibitor 0.19 in celiac disease. Physiol Res 67:613-622. DOI: 10.33549/physiolres.933876

Tundo S, Lupi R, Lafond M, Giardina T, Larré C, *et al*. (2018) Wheat ATI CM3, CM16 and 0.28 allergens produced in *Pichia pastoris* displays a different eliciting potential in food allergy to wheat. Plants 7:101. DOI: 10.3390/plants7040101

Zevallos VF, Raker V, Tenzer S, Jimenez-Calvente C, Ashfaq-Khan M, *et al*. (2017) Nutritional wheat amylase-trypsin inhibitors promote intestinal inflammation via activation of myeloid cells. Gastroenterology 152:1100-1113.e12. DOI: 10.1053/j.gastro.2016.12.006

Appendix

Extraction and purification of ATIs from a natural source

nATIs were extracted from whole-grain wheat flour (*T. aestivum* cv. 'Arnold', particle size <0.2 mm). After defatting with *n*-hexane, nATIs were extracted in a mixture of chloroform and methanol (CM). Insoluble components were separated by centrifugation before the CM supernatant was evaporated. The solid residue was re-suspended in saline (0.2 M PBS, pH 7.5) to extract the nATIs. After centrifugation, the nATI-containing supernatant was further purified by gel filtration chromatography (GF) using an Agilent 1260 Infinity II LC system (Agilent, Santa Clara, USA) equipped with a ShodexTM PROTEIN KW-802.5 gel filtration column (Resonac America, Inc., New York, USA). Fractions containing proteins with the desired molecular weight (11–16 kDa, confirmed by SDS-PAGE) were pooled and concentrated using centrifugal filters with a cut-off of 10 kDa (Merck Millipore, Burlington, USA). Finally, affinity purification of nATIs was performed with TPCK-trypsin immobilized on magnetic beads (Takara Bio Europe, Saint-Germain -en-Laye, France). The clean-up procedure was performed according to the manufacturer's instructions.

Heterologous expression of ATIs in E. coli and P. pastoris

Methodological details for the heterologous expression of recombinant ATIs proteins in *Escherichia coli* and *Pichia pastoris* are given in Table 1. Expression in *E. coli* systems was performed after induction with IPTG (isopropyl β-D-1-thiogalactopyranoside) at 30°C in TB medium (Terrific Broth). Cells were harvested and sonicated to release the expressed proteins to the medium. However, SDS-PAGE analysis revealed the presence of the target proteins in the insoluble culture residues that were dissolved in urea buffer. Since the proteins expressed in *E. coli* systems in this study were His_6-tagged, their purification was performed by affinity chromatography using HisTrap^{TM} FF crude columns (Cytiva – Global Life Sciences Solutions Austria, Pasching, Austria). Urea extracts containing denatured rATIs were re-buffered on the column with saline prior to elution with imidazole. Nevertheless, long-term storage of these proteins in PBS resulted in precipitation. For the production of recombinant ATIs in *P. pastoris*, expression was induced by incubating cultures in BMMY medium (Buffered Methanol-complex Medium) at 25°C. Culture supernatants containing secreted proteins were recovered by centrifugation and subjected to purification by ion-exchange chromatography (IEX) using diethylaminoethy cellulose (DEAE-C) and GF. The presence and purity of the targeted ATI proteins were confirmed by SDS-PAGE, MALDI-TOF MS, and LC-MS/MS.

Table 1 Expression details for the heterologous production of recombinant ATI proteins (rATIs) in *Escherichia coli* and *Pichia pastoris*

Protein	UniProt no.	Vector	Tag	Expression system
0.28 (*E. coli*)	P01083	pET-DUET1	N-terminal His_6 tag	*E. coli* T7
CM3 (*E. coli*)	P17314	pET-DUET1	N-terminal His_6 tag	*E. coli* Shuffle
0.19 (*P. pastoris*)	P01085	pPICZαC	-	*P. pastoris* SuperMan5
CM1 (*P. pastoris*)	P16850	pPICZαC	-	*P. pastoris* SuperMan5
CM3 (*P. pastoris*)	P17314	pPICZαC	-	*P. pastoris* SuperMan5

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

From farm to fork: Evaluation of the large panels of different emmer and einkorn varieties on agronomic, flour and quality traits in comparison to common wheat, spelt and durum

Muhammad AFZAL[1], Franz PFLEGER[2], Norbert HUINTJES[2], Melanie RUHRLÄNDER[2], Wessam AKEL[3], Friedrich LONGIN[1]

[1] University of Hohenheim, State Plant Breeding Institute, Fruwirthstr. 21, 70599 Stuttgart, Germany

[2] DIGeFa GmbH, Detmold Institute for Grain and Fat Analysis, Schützenberg 10, 32756 Detmold, Germany

[3] Strube Research GmbH & Co. KG, Hauptstr. 1, 38387 Söllingen, Germany

(✉) friedrich.longin@uni-hohenheim.de

Abstract

Wheat (*Triticum* L.) is one of the most important staple foods that provides 20% of the daily intake of dietary protein together with dietary fibre, minerals, and vitamins. Most of the production is contributed by common wheat (*T. aesti*vum) and durum (*T. durum*). Ancient wheat species (*i.e*. emmer and einkorn) have been utilized as food for thousands of years, but are currently cultivated only on a small-scale confined to specific regions. Currently, consumers are increasingly interested in products made from ancient grains. Therefore, using two large panels of emmer and einkorn varieties, we aimed to (i) assess the genetic variability of agronomic and important quality traits across varieties, (ii) develop suitable milling and baking trials, (iii) investigate rapid methods for quality determination, (iv) develop guidelines for processing of grains into premium products, and (v) discover the genetics behind important traits.

In the emmer project, we evaluated 143 different varieties of winter emmer with check varieties of common wheat ('Genius', 'Julius'), spelt ('Zollernspelz', 'Franckenkorn'), durum ('Sambadur', 'Wintergold') and einkorn ('Terzino') in multi-environment trials. In the einkorn project, 148 einkorn varieties together with two common wheat varieties ('Genius', 'Julius') were included in the multi-environment trials. Additionally, for einkorn, separate field trials were performed specifically to study frost tolerance and vernalization requirement.

Several traits such as raw yield, grain yield, plant height, heading date, lodging, resistance to different diseases, grain protein content and SDS sedimentation value were recorded. Moreover, DIGeFa developed a standard milling test for emmer and einkorn. The aim was to achieve the highest possible flour yield. Using these flours, various dough properties were then evaluated using the Mixolab® and Extensograph® devices. In addition, a standard baking test for emmer and einkorn was developed. Based on the Mixolab curves, we identified that samples should be kneaded shorter than common wheat or spelt.

The statistical analysis was performed using ASReml® R software (VSNi, Hemel Hempstead). Genotyping was done by genotyping-by-sequencing (GBS) at Diversity Arrays Technology (Bruce, ACT, Australia). Association mapping was conducted using the BLINK method in the R package GAPIT (Lipka *et al*., 2012).

Although emmer and einkorn are cultivated only on a very small scale, the variability across varieties was high for all evaluated traits. The raw yield for emmer and einkorn ranged from 34 to 80 $dt \cdot ha^{-1}$ and 38 to 61 $dt \cdot ha^{-1}$, respectively. Compared to common wheat, emmer and einkorn showed on average a lower grain yield, higher protein content, lower SDS sedimentation volume, lower dough stability, and lower dough extensibility. Emmer has a higher risk to lodging, higher yellow rust infection, higher water uptake and lower starch retrogradation compared to common wheat. Thus, the different dough properties of emmer and einkorn must be considered for baking trials and the assessment of baking quality should include loaf volume and the height/width ratio of the bread. SDS sedimentation volume correlated quite well with baking quality, while protein content did not. Hence, baking quality can be estimated quickly by measuring the SDS sedimentation value. Notably, the protein content of einkorn varieties says nothing about their baking quality. Currently, we consider the processing quality of einkorn varieties to be less important than its improved agronomic performance. This is because the bakers can already achieve good and tasty baked goods with long shelf life by adapting recipes for einkorn. Moreover, our findings showed that, for emmer and einkorn, careful kneading, longer dough processing and stabilization of the doughs are particularly important. Similar to emmer, the einkorn varieties differ significantly in agronomic characteristics as well as in processing quality. The choice of variety is therefore of particular importance. In the current supply chain, the selection of varieties should mainly be aimed at achieving high yield with low farming risk, whereas bakers should adapt recipes to account for emmer- or einkorn-specific properties for producing premium products.

Afzal M, Pfleger F, Huintjes N, Ruhrländer M, Akel W, *et al*. (2023) From farm to fork: evaluation of the large panels of different emmer and einkorn varieties on agronomic, flour and quality traits in comparison to common wheat, spelt and durum. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 51-52. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

Genome-wide association mapping in einkorn revealed interesting QTL for two important traits, *i.e*., frost tolerance and vernalization requirement. For frost tolerance, QTL were discovered on chromosomes 1A, 2A, 4A, 5A, and 6A. Particularly, two QTL on chromosome 2A, not yet found in common wheat, seem to play an important role, as they explain 62% and 19% of the genetic variance, respectively. The largest QTL for vernalization requirement found on chromosome 5A explained 45% of the genetic variance. In common wheat, chromosome 5A harbours the candidate gene *Vrn1* for vernalization requirement. However, the diagnostic marker used from common wheat could not differentiate between the einkorn varieties. Further studies are warranted to demonstrate whether the QTL found in our study is a yet unknown allele of *Vrn1* or another gene. In particular, the markers for frost tolerance are interesting, since it is a difficult trait to phenotype in the field. In addition, the two QTL for frost tolerance on chromosome 2A could also be of particular importance for other wheat species. In common wheat, there are varieties that have no need for vernalization, but are not frost-tolerant (spring wheat), varieties that have high vernalization requirements and high frost tolerance (winter wheat) and varieties that have high vernalization requirements and no frost tolerance (not useful), but there are no varieties that have no need for vernalization but are still very frost-tolerant, as in einkorn. We hypothesize that the two QTL on chromosome 2A confer vernalization-independent frost tolerance. Nonetheless, this warrants further investigations and, if necessary, their introgression in common wheat and durum.

In summary, the research approach in our two projects considering the whole supply chain proves to be of central importance to sustainably support the establishment of the ancient crops. The developed methods, in particular the milling and baking test, can now be used as the gold standard in the evaluation of emmer and einkorn samples. Other laboratory methods such as the mixolab and extensograph can help to better assess the baking and dough properties of emmer and einkorn. Breeding can be assisted and accelerated based on the estimated quantitative parameters and the discovered QTL.

Keywords

Ancient wheat · bread quality · breeding · supply chain · QTL · *Triticum*

Acknowldedgements

The emmer project was funded by the *Ministerium für ländlichen Raum und Verbraucherschutz (MLR) im Rahmen des Sonderprogramms zur Stärkung der biologischen Vielfalt des Landes Baden-Württemberg*. The einkorn project was funded by the *Zentrale Innovationsprogramm Mittelstand* of the German Federal Ministry for Economic Affairs and Energy based on a resolution of the German Federal Parliament (FKZ16KN068825, 16KN068826, 16KN068827). We thank our partners DIGeFa, Strube Research, Pflanzenzucht Oberlimpurg, and Südwest-deutsche Saatzucht for their excellent cooperation.

References and further reading

Afzal M, Longin F, Pfleger F, Huintjes N, Rührländer M (2022) Mahl-, Teig- und Backeigenschaften von 143 verschiedenen Emmersorten. Universität Hohenheim, Landessaatzuchtanstalt, Arbeitsgruppe Weizen. [https://weizen.uni-hohenheim.de/fileadmin/einrichtungen/lsa-weizen/Dateien/Emmer_MLR_Quali1_final.pdf; accessed 30 Jan 2023]

Lipka AE, Tian F, Wang Q, Peiffer J, Li M, *et al*. (2012) GAPIT: genome association and prediction integrated tool. Bioinformatics 28:2397-2399. DOI: 10.1093/bioinformatics/bts444

Longin F, Dehn S (2022) Kleine Arten, große Unterschiede: Dinkel, Emmer und Einkorn im Backtest. Berichte über Landwirtschaft 100 (2): 7 p. DOI: 10.12767/buel.v100i2.420

Longin F, Ringer L (2022) Emmer - Qualität und Agronomie im Vergleich zu Dinkel. Berichte über Landwirtschaft 100 (1): 10 p. DOI: 10.12767/buel.v100i1.405

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Suitability of micro baking test to select for quality of organic einkorn in comparison to different parameters over three years

Yazdan VAZIRITABAR[1], Karl-Josef MÜLLER[1]

[1] Cultivari gGmbH, Cereal Breeding Research Darzau, Hof Darzau 1, 29490 Neu Darzau, Germany

(✉) office@cultivari.de

Abstract

This study was conducted to evaluate the baking quality of organic einkorn. A micro baking test with a long-term processing was carried out in parallel with some wheat quality parameters and the results were compared thoroughly with the micro baking test results. A set of 42 einkorn varieties and breeding lines were analyzed over three years (2020-2022), covering two locations. The analysis of variances showed significant differences in baking volume for genotypes and locations. Wheat quality parameters (*e.g.*, falling number, sedimentation value, gluten index) traditionally used for the prediction of baking quality were not correlated with micro baking test volume. Only grain protein content (hulled and hull-less einkorn) was weakly (r=0.54**) correlated with the micro baking test volume.

Keywords

Baking volume · falling number · gluten index · sedimentation test · *Triticum monoccocum*

Introduction

Einkorn flours are characterized by a sticky dough consistance at kneading and lower water uptake compared to wheat. Regarding the soft consistency of gluten in einkorn, baking with long-term processing is recommended for better preserving the high carotenoid content despite a longer dough contact with atmospheric oxygen (Antognoni *et al.*, 2017). Another study showed that longer fermentation time can decrease wheat related health disorders (Ziegler *et al.*, 2019). The aim of the study was to verify the suitability of the micro baking test for breeding decisions. In addition, it was evaluated which grain quality parameters help in the selection of breeding lines. For establishing a micro baking test for einkorn, the special baking behaviour had to be taken into account, *i.e.*, the sticky dough consistance at kneading and the low water uptake in comparison to common wheat.

Material and methods

A total of 42 samples (10 hull-less and 32 hulled einkorn) were tested over three years at a location near Koehlingen (2020-2022) and over two years in Hohenlohe (2021-2022). The indirect parameters falling number (ICC Standard Nr.107/1, AACC Method 56-81B), thousand grain weight, gluten-index (Newport Scientific Method Nr. 21, vers. 2; time reduced to 3 minutes), SDS-sedimentation with 10 mL according to Mc Donald (1985) and grain protein determined by NIRS were correlated with the micro-baking-test volume.

The micro baking test was done with 20 g flour, 2.5% yeast, 3% salt and an individual volume of water, estimated by the Micro-Dough-Lab (Perten, Germany). All ingredients were mixed and kneaded by hand for 3 minutes. The dough rested for 23 hours at 4°C. It was fermented at 30°C for 45 minutes and afterwards baked at 190°C for 17 minutes. After cooling, the loaf volume was measured in mL per 20 g flour according to AACC 10–05.01. The yield of hulled einkorn varieties was calculated including the hulls.

Results and discussion

The analysis of variance showed significant differences in baking volume among the investigated locations ($p < 0.001$). Pairwise comparisons showed that Hohenlohe 2022, Hohenlohe 2021 and Koehlingen 2020 differed significantly from Koehlingen 2021 and Koehlingen 2022. The analysis of variance across all years and locations also showed significant differences ($p < 0.001$) between lines. Among varieties, only the hull-less einkorn breeding line DZM1034i31 with a mean value of 68.2 mL·20 g^{-1} flour significantly differed from the other varieties and breeding lines, respectively. A high baking volume was achieved by the hulled einkorn strain DZM1005d with an average of 64.4 mL·20 g^{-1} flour. 'Enkidu' and the hull-less einkorn strain DZM1124f realized 63.4 mL·20 g^{-1} flour on average.

The hulled variety 'Enkidu' realized a far above-average yield (32.3 dt·ha^{-1}) and baking volume, which is particularly striking (Fig. 1). The breeding line DZM1005d with its noticeably high baking volume but only medium high yield (26.6 dt·ha^{-1}) lies also outside the

Vaziritabar Y, Müller KJ (2023) Suitability of micro baking test to select for quality of organic einkorn in comparison to different parameters over three years. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 53-54. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

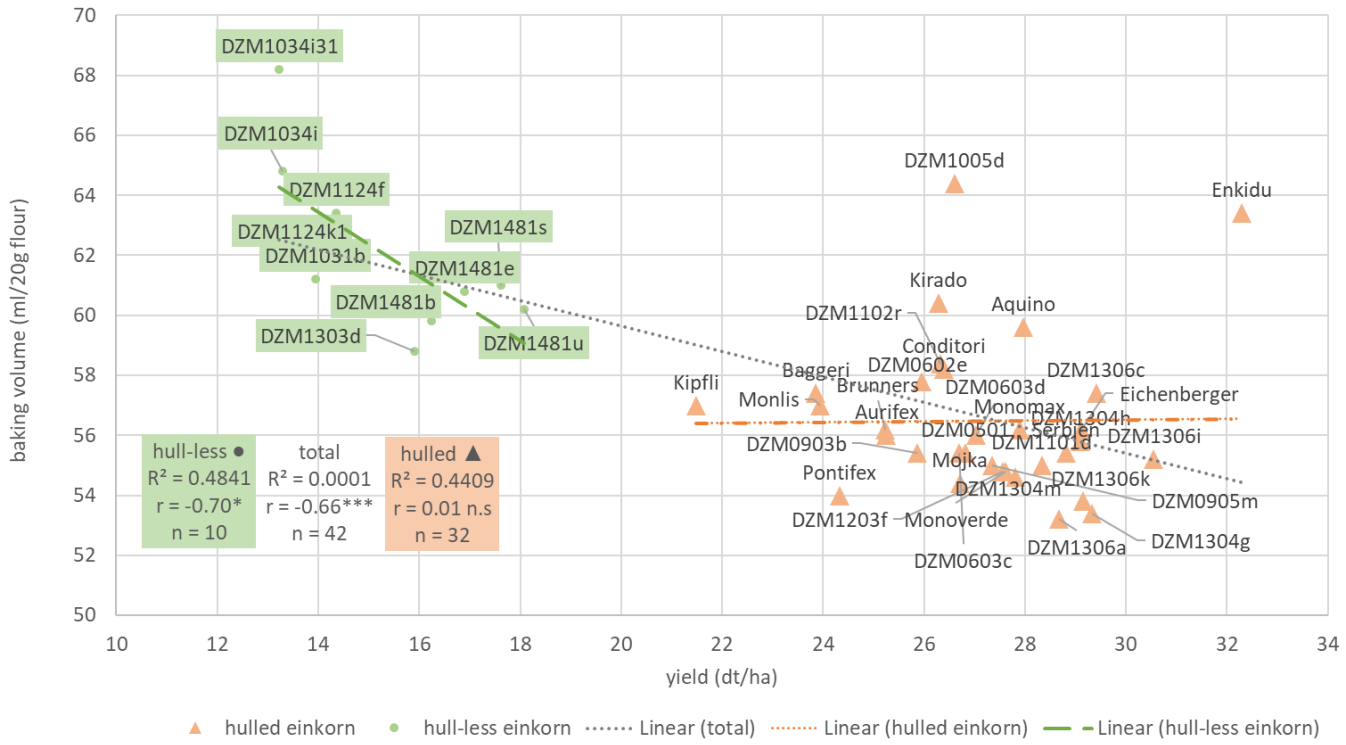

Figure 1 Relationship between yield and baking volume in hulled and hull-less einkorn genotypes.

cluster of the other genotypes. Breeding line Kirado (61 mL·20 g^{-1} flour) and breeding line Aquino (60 mL·20 g^{-1}) showed good baking volumes with medium yields (26.4 and 28 dt·ha^{-1}, respectively). The hull-less einkorn lines showed a decreasing trend in baking volume with increasing yields, which is less pronounced in the hulled einkorn lines.

The three locations with higher baking volumes (Hohenlohe 2021, Hohenlohe 2022 and Koehlingen 2020) were positively correlated with each other, as the locations with low baking volumes were correlated with each other (Koehlingen 2021 and Koehlingen 2022) (Table 1). These results showed the reproducibility of the baking results. At Hohenlohe the baking volume was negatively correlated with falling number in both years (2021: r = -0.57***; 2022: r = -0.43**). At Hohenlohe in 2021 the gluten-index was positively correlated (r = 0.35*) with baking volume and thus showed a slight influence on the total baking volume. At Koehlingen in 2020 and 2022, baking volumes were generally low. In addition, the SDS value was correlated with the baking volume in 2020 (r = 0.43*) as well as in 2021 (r = 0.50**). Although a positive correlation (r = 0.43*) was found between protein content and baking volume at Koehlingen 2021, there were no significant correlations between protein content and baking volume in general among hulled and hull-less einkorn grains. For hulled einkorn a low correlation (r = 0.35*) was found between SDS-sedimentation and baking volume. None of the parameters traditionally used for indirect quality determination in common wheat was suitable for the prediction of baking volume in einkorn varieties. Contradictionary, the micro baking test showed a suitable potential for differentiation of breeding lines and varieties. It could be a useful test method in selection for better baking quality of einkorn.

Table 1 Correlation of baking volumes between the test environments (Hoh, Hohenlohe; Koe, Koehlingen; n.s., not significant; *, p<0.05; **, p<0.01; ***, p<0.001)

Environment	Hoh 2021	Hoh 2022	Koe 2020	Koe 2021
Hoh 2022	0.31*			
Koe 2020	0.63***	0.41**		
Koe 2021	n.s.	n.s.	0.30*	
Koe 2022	n.s.	n.s.	n.s.	0.53***

Acknowledgements

The project was supported by the German Federal Ministry for Food and Agriculture (BMEL) based on a decision of the parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the federal program for ecological farming (BÖL Project-ID 2815OE054).

References

Antognoni F, Mandrioli R, Bordoni A, Di Nunzio M, Viadel B, *et al.* (2017) Integrated evaluation of the potential health benefits of einkorn-based breads. Nutrients 9:11. DOI: 10.3390/nu9111232

McDonald CE (1985) Sodium dodecylsulfat sedimentation test for durum wheat. Cereal Foods World 30: 674-677.

Ziegler K, Neumann J, Liu F, Fröhlich-Nowoisky J, Cremer C, *et al.* (2019) Nitration of wheat amylase trypsin inhibitors increases their innate and adaptive immunostimulatory potential *in vitro*. Front Immunol 9:3174. DOI: 10.3389/fimmu.2018.03174

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Desirable characters of triticale and winter pea for organic mixed cropping

Karl-Josef MÜLLER[1]

[1] Cultivari Getreidezüchtungsforschung Darzau gGmbH, Hof Darzau 1, 29490 Neu Darchau, Germany

(✉) k-j.müller@cultivari.de

Abstract

Twenty different triticale, representing a broad spectrum of morphological types, were grown in five trials as mixed cropping with winter pea cv. 'Kolinda' under organic farming conditions in Germany. No correlations could be found between grain yield of the pea component and grain yield, plant height and leaf width during juvenile stages, plant height and heading date of triticale. However, combinations of high grain yield of pea and high total yield could always be found among the intermediate types of triticale with respect to heading date, plant height and leaf width in early spring. Triticale varieties should neither be too early or late, have too narrow or broad leaves, nor have a too prostrate or erect growth type during juvenile development. Breeding for suitability of winter triticale for mixed cropping with pea should look for intermediate types, which then should be tested for yield in specific trials with a representative winter pea.

Keywords

Breeding · competitiveness · ear emergence · juvenile growth · *Pisum sativum* · yield · *×Triticosecale*

Introduction

Mixed cropping of winter pea with winter triticale can reduce the risk of low yield if peas are damaged by low winter temperatures or by stem diseases under cold weather in spring. In most cases triticale can compensate the loss of peas, which avoids the recultivation of the field in spring with another crop. The aim of this study was to compare different types of triticale for their suitability in mixed cropping with winter pea cv. 'Kolinda'. We focused on the question which characters could be useful to get a high yield from both crops with an emphasis on pea.

Material and methods

The project started with 100 winter triticale varieties and breeding lines in 2019, representing a broad spectrum of morphological types. In the second year, the number of genotypes was reduced to 35 by eliminating those with susceptibility to leaf diseases, in particular yellow rust, and by taking only a few representatives of similar morphological types with an emphasis on better yielding ones. In a similar way, the number of entries was reduced to 20 for the 2021/22 season. All trials were grown under organic farming conditions in all years near Köhlingen (53.216 N, 10.836 E) on sandy loam after spring oat. In the third year, the field trials were additionally carried out near Rendsburg (54.346 N, 9.730 E) and in Hohenlohe (49.247 N, 10.040 E). Seed density of triticale was 120 seeds per m² in the first and 150 seeds per m² in the second and third year. Winter pea 'Kolinda' was grown with a seed density of 70 seeds per m² in the first two years, and with 60 seeds per m² in the third year. As in previous trials short peas were significantly suppressed by triticale and ripened too early compared to triticale and very tall normal-leaf types always pulled down triticale before maturity, 'Kolinda' was used as a representative for medium-tall, white flowering, semi-leafless types, which were found to be most suitable for combination with winter triticale (Müller, 2021). Juvenile plant height was measured at BBCH 31 only in Köhlingen, where also leaf width was measured about 1-2 days after juvenile plant height. Ear emergence was noticed as day in June. Plant height was measured before harvest. In the first two seasons also the number of shoots after winter was counted. However, this trait was not of any added value. After harvest with the plot combine, the samples were dried, weighed and the grains were separated according to crop species. Triticale grain yield was calculated as total plot yield minus the respective pea yield. All trials were done with two replications and statistical analyse via the nearest neighbour method according to Schwarzbach (1984).

Results and discussion

In all trials there were no correlations between grain yield of triticale and grain yield of pea. Across the five test environments, grain yield of the triticale component ranged from 87% to 114% and for the pea component from 76% to 124% relative to the component's mean across all trials (Fig. 1). Analysis of variance allowed to postulate an influence of triticale varieties on total plot yield and on triticale grain yield, but also, except for Rendsburg, on grain yield of the pea component. There was an influence of test environment on the yield of triticale and pea, and an genotype by environment interaction for total and triticale yield, but not for the pea yield.

Plant height of triticale at BBCH 31 showed a low correlation (r = 0.45*) to yield of triticale and leaf width (r = 0.52*). However, neither traits showed a significant correlation to pea yield. For ear emergence and plant height no significant correlations with the

Müller KJ (2023) Desirable characters of triticale and winter pea for organic mixed cropping. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21.-23. November, Raumberg-Gumpenstein, pp 55-58. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

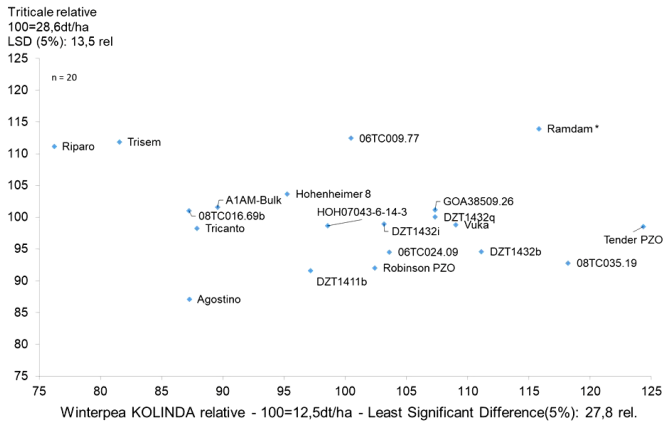

Figure 1 Relative yield of winter pea cv. 'Kolinda' in mixed cropping with 20 triticale genotypes. Grain yields of the two crop species were expressed relative to the mean of the single test environments and finally averaged over all five test environments.

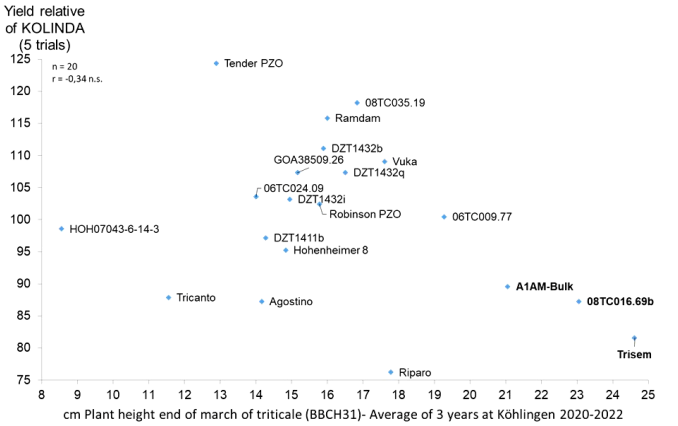

Figure 2 Relative yield of winter pea cv. 'Kolinda' in mixed cropping with 20 triticale genotypes in relation to triticale plant height at BBCH 31. Data for the pea crop are means across 5 test environments, triticale plant height was measured only in Köhlingen (*n*=3).

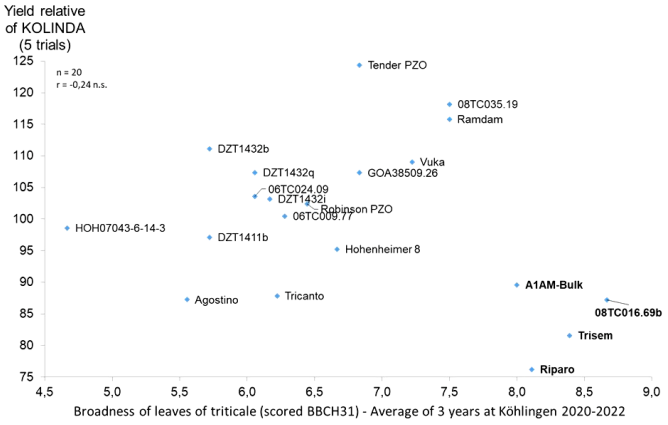

Figure 3 Relative yield of winter pea cv. 'Kolinda' in mixed cropping with 20 triticale genotypes in relation to triticale leaf width at BBCH 31. Data for the pea crop are means across 5 test environments, triticale plant height was measured only in Köhlingen (*n*=3).

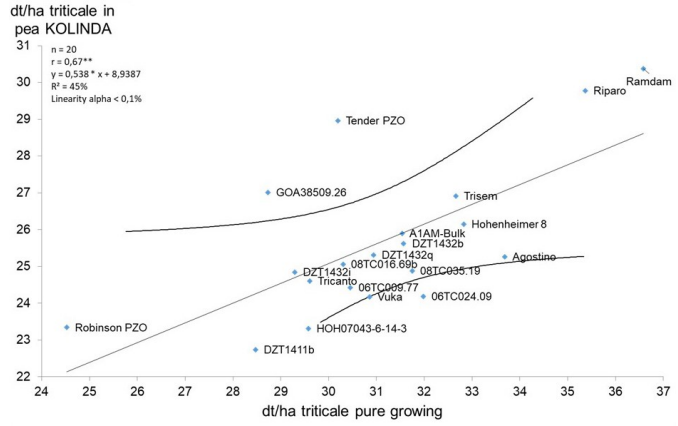

Figure 4 Regression of triticale grain yield in mixed cropping with winter pea cv. Kolinda on triticale grain yield in pure growing in test environment Köhlingen 2021/22.

yield of triticale or pea could be found. However, a significant correlation was observed between leaf width and plant height at BBCH 31 (r = 0,88***) and between heading date and plant height (r = -0,66**) and leaf width (r = -0.66**), respectively. Early maturing triticale seem to have always broad leaves and a vigorous erect growth in spring, whereas very late maturing genotypes more often have narrow leaves and a prostrate growth in spring. An influence of evaluated traits on yield is visible when the data are plotted as scatter plots of the respective trait and grain yield, separated for triticale and pea. In this case, highest pea yields were realized for triticale varieties with intermediate performance for plant height and leaf width at juvenile growth (Fig. 2 & Fig. 3). Across all test environments, cvs. 'Ramdam' and 'Tender PZO' showed the best performance with respect to pea yield in combination with triticale yield. Both triticale cultivars showed no extraordinary characters and provided the co-cultivated pea enough space for development. It was obvious that vigorous growing triticale genotypes with broad leaves were highly competitive for light and triticale genotypes with a slow juvenile development provided not enough protection for the peas during early spring. It seems that a similar fast development of the pea and triticale crop with triticale being a small step ahead stimulates the development of the pea crop. Moreover, competitiveness or complementing between the crops in the root system size could influence the mixed growing.

In Köhlingen 2021/22 a comparison of triticale grain yield under mixed cropping and pure growing with 300 seeds per m² was done. In this experiment a significant correlation (r = 0.67**) was observed. This result showed the general high influence of the yield potential of the triticale genotype, but also that in mixed cropping with winter pea and using a seed density of only 150 seeds per m² the response is not always linear for all genotypes (Fig. 4).

Besides all the measured traits it became obvious during the practical work of the study, that a triticale suitable for mixed cropping with pea also should have a very good threshability as the combine has to be adjusted for the pea crop. If grain shape is more rye-like, meaning longer and narrower compared to wheat, peas can be separated easier. Ripening of triticale should not occur much later then the ripening of the peas, because otherwise pea yield is reduced due to pod dehiscence of already mature peas.

Conclusion

For developing new varieties of triticale for mixed cropping with winter peas, all extraordinary morphological types could be discarded. To find the best suitable triticale genotype, it cannot be avoided to test the yield potential of triticale genotypes in practical field experiments with a suitable winter pea of medium to tall plant height and relatively late maturity.

Acknowledgements

The trials were made possible by financial support of Zukunftsstiftung Landwirtschaft, Bochum, and additional support from Demeter-im-Norden, Lüneburg.

References and further reading

Annicchiarico P, Collins RP, De Ron AM, Firmat C, Litrico I, *et al*. (2019) Do we need specific breeding for legume-based mixtures? Adv Agron 157:141-215. DOI: 10.1016/bs.agron.2019.04.001

Annicchiarico P, Nazzicari N, Notario T, Monterrubio Martin C, Romani M, *et al.* (2021) Pea breeding for intercropping with cereals: Variation for competitive ability and associated traits, and assessment of phenotypic and genomic selection strategies. Front Plant Sci 12:731949. DOI: 10.3389/fpls.2021.731949

Bedoussac L, Journet E-P, Hauggaard-Nielsen H, Naudin C, Corre-Hellou G, *et al*. (2015) Ecological principles underlying the increase of productivity achieved by cereal-grain legume intercrops in organic farming. A review. Agron Sust Dev 35:911-935. DOI: 10.1007/s13593-014-0277-7

Haug B, Messmer MM, Enjalbert J, Goldringer I, Forst E, *et al*. (2021) Advances in breeding for mixed cropping – Incomplete factorials and the producer/associate concept. Front Plant Sci 11:620400. DOI: 10.3389/fpls.2020.620400

Haug B, Messmer M, Goldringer I, Forst E, Mary-Huard T, *et al*. (2021) Genetic drivers for mixture performance in pea and barley. Aspects Appl Biol 146:165-167.

Müller KJ (2021) Wintererbsen-Sortenvergleiche für den Ökologischen Anbau in Niedersachsen. Cultivari, Darzau. [https://www.cultivari.de/fileadmin/user_upload/Projekte/Wintererbsen/CultivariErbsensortenvergleicheBis2021.pdf; verified 24 Jan 2023]

Quendt U, Müller K-J (2011) Winter pea breeding in intercropping and mixtures with cereals. In: Lammerts van Bueren ET, *et al*. (Eds), Proc ECO-PB 10 y Anniv Conf, Organic plant breeding: What makes the difference?, 3-4 Nov, Frankfurt/Main, pp 24-25.

Schwarzbach E (1984) A new approach in the evaluation of field trials: the determination of the most likely genetic ranking of varieties. Vorträge für Pflanzenzüchtung 6:249-259.

Appendix

Supplementary Table S1 Means for morphological traits and grain yield for winter triticale and winter pea in mixed cropping

	Triticale										winter pea 'Kolinda'				
	3 year averages				Grain yield (dt·ha^{-1})						Grain yield (dt·ha^{-1})				
Triticale genotype	Leaf width (mm) at BCH31	Plant height (cm) at BBCH 31	Plant height (cm) at maturity	Heading date (day of June)	Köhlingen 2019/20	Köhlingen 2020/21	Köhlingen 2021/22	Rendsburg 2021/22	Hohenlohe 2021/22	Köhlingen 2021/22 pure stand	Köhlingen 2019/20	Köhlingen 2020/21	Köhlingen 2021/22	Rendsburg 2021/22	Hohenlohe 2021/22
06TC009.77	6.3	19	98	14	14.6	18.6	24.4	49.2	46.4	30.5	9.3	18.6	18.6	7.5	6.9
06TC024.09	6.1	14	99	14	11.8	10.5	24.2	46.4	47.3	32.0	13.4	23.0	18.3	5.0	7.0
08TC016.69b	8.7	23	101	13	12.9	14.3	25.1	45.4	44.4	30.3	11.6	17.1	15.1	4.9	5.8
08TC035.19	7.5	17	98	16	9.5	13.3	24.9	42.9	44.1	31.7	15.9	21.7	15.8	10.5	6.3
A1AM-Bulk	8.0	21	107	13	12.6	14.3	25.9	42.2	48.7	31.5	7.3	16.6	14.7	9.1	5.1
Agostino	5.6	14	86	19	11.6	9.4	25.3	39.0	40.6	33.7	11.4	20.7	18.5	0.4	8.0
DZT1411b	5.7	14	101	14	12.4	11.5	22.7	38.6	46.0	28.5	13.4	18.8	15.2	6.6	5.9
DZT1432b	5.7	16	100	17	14.3	8.7	25.6	42.7	46.4	31.6	14.7	19.4	16.4	9.1	6.5
DZT1432i	6.2	15	104	19	12.8	11.4	24.8	47.2	48.7	29.3	11.6	22.1	14.6	7.3	7.3
DZT1432q	6.1	17	103	17	13.8	11.8	25.3	43.4	49.2	30.9	13.3	23.1	15.1	8.4	6.2
GOA38509.26	6.8	15	104	17	11.1	14.2	27.0	45.8	47.8	28.7	12.5	22.6	14.9	9.8	5.4
HOH07043-6-14-3	4.7	9	113	20	11.9	11.7	23.3	48.9	51.6	29.6	12.7	24.1	16.5	6.0	5.1
Hohenheimer 8	6.7	15	102	17	13.9	12.8	26.2	51.0	44.6	32.8	10.9	22.7	15.4	6.2	5.7
Ramdam	7.5	16	111	16		15.1	30.4	42.9	59.0	36.6		26.0	16.4	8.0	7.6
Riparo	8.1	18	96	15	14.2	16.1	29.8	48.6	44.6	35.4	9.8	18.3	14.3	3.0	5.1
Robinson PZO	6.4	16	98	17	9.9	12.7	23.3	41.2	47.5	24.5	14.3	23.2	16.4	4.8	7.1
Tender PZO	6.8	13	117	17	12.0	11.7	29.0	37.8	51.9	30.2	13.4	27.0	16.0	10.4	8.1
Tricanto	6.2	12	112	15	14.1	12.2	24.6	41.9	45.9	29.6	12.7	17.1	16.1	3.0	7.0
Trisem	8.4	25	115	14	15.0	17.5	26.9	42.3	50.4	32.7	9.2	17.8	15.3	2.6	7.4
Vuka	7.2	18	100	16	13.9	12.0	24.2	48.5	42.0	30.9	11.3	24.8	18.3	7.6	6.8
Average	6.7	16	103	16	12.8	13.0	25.6	44.3	47.4	31.0	12.0	21.2	16.1	6.5	6.5

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Assessing durum wheat for organic farming

Luca BONFIGLIOLI[1], Ieva URBANAVIČIŪTĖ[1], Mario Augusto PAGNOTTA[1]

[1] Department of Agricultural and Forest Sciences, Tuscia University, via S. C. de Lellis, snc, 01100 Viterbo, Italy

(✉) pagnotta@unitus.it

Abstract

Sustainability in agroecosystems is ensured by a high degree of diversity and the capability to tolerate external inputs without significant structural and functional changes. The most important challenges of organic farming include the production of sufficient, healthy and affordable food, the reduction of pollution, the adaptation to climate change, and the protection of biodiversity. Since the European organic system is growing consistently year by year, with an annual increase of 5.9% in organic farmland and a growth of 8% of the organic market, more organic breeding programmes are needed for all agricultural crops. The objective of this work was the evaluation of some durum wheat accessions for a final selection of the most suitable genotype for organic production, with a consequent opportunity to create a new ideotype that allows less input (such as fertilizers and pesticides) with a satisfying level of grain yield and quality.

A total of 41 accessions of different origins, *i.e*. the Mediterranean Basin and Central Europe, were evaluated at the experimental farm 'Nello Lupori' of Tuscia University, Viterbo, during the season 2021/2022. The plot trial was conducted with three replications, sowing density was the same as used by organic farmers in the local area. Pre-harvest and post-harvest traits were recorded during the vegetation period.

The statistical analysis of ground cover, grain yield, and protein content showed a big difference between the genotypes developed in the two regions of origin. Despite that genotypes developed in the Mediterranean Basin showed a more erected growth habit than those from Central Europe, they had a higher rate of ground-cover (Fig. 1). Due to the particular dry year with only 323 mm rainfall between September 2021 and August 2022, a protein content >15% was recorded for all genotypes, but it was generally higher in the Central European germplasm with a maximum of 21.4%. Interestingly, the dry season didn't affect grain yield of all genotypes, probably because high rainfall in fall 2021 provided enough water reserves for the overall season. Most Mediterranean accessions showed high grain yield and yield stability, statistically higher than the Central European accessions.

The higher ground cover observed for Mediterranean genotypes indicate them as good candidates for the creation of new varieties with a high potential of weed competitiveness. Genotypes from Central Europe are an optimal resource of high protein content which makes them interesting for organic agriculture that focuses on end-use quality, thus giving emphasis on grain quality. Mediterranean genotypes showed a better adaptation to drought conditions resulting in higher grain production. Crosses between genotypes from the two different areas are currently under evaluation with the aim to create a new ideotype for organic farming considering the best characteristics of the parents in the selection process.

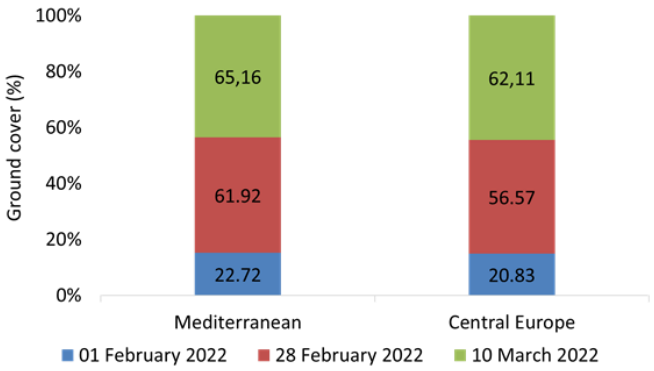

Figure 1 Mean ground cover rate of Mediterranean and Central European durum wheat accessions at three different dates of measurement.

Keywords

Organic breeding · protein content · quality · *Triticum durum* · weed competitiveness

Acknowledgements

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

Further reading

Brokensha D, Warren DM, Wertner O (eds) (1980) Indigenous knowledge systems and development. University Press of America, Lanham, MD.

Bonfiglioli L, Urbanavičiūtė I, Pagnotta MA (2023) Assessing durum wheat for organic farming. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 59-60. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

Campiglia E, Mancinelli R, De Stafanis E, Pucciarmati S, Radicetti E (2015) The long-term effects of conventional and organic cropping systems, tillage managements and weather conditions on yield and grain quality of durum wheat (*Triticum durum* Desf.) in the Mediterranean environment of Central Italy. Field Crops Res 176:34-44. DOI: 10.1016/j.fcr.2015.02.021

Lammerts van Bueren ET, Jones SS, Tamm L, Murphy KM, Myers JR, *et al*. (2011) The need to breed crop varieties suitable for organic farming, using wheat, tomato and broccoli as examples: A review. NJAS Wageningen J Life Sci 58:193-205. DOI: 10.1016/j.njas.2010.04.001

Rahmann G, Ardakani MR, Bàrberi P, Boehm H, Canali, *et al*. (2017) Organic Agriculture 3.0 is innovation with research. Organic Agric 7:169-197. DOI: 10.1007/s13165-016-0171-5

Stagnari F, Onofri A, Codianni P, Pisante M (2013) Durum wheat varieties in N-deficient environments and organic farming: a comparison of yield, quality and stability performances. Plant Breed 132:266-275. DOI: 10.1111/pbr.12044

Willer H, Trávníček J, Meier C, Schlatter B (eds) (2021) The world of organic agriculture 2021 - Statistics and emerging trends. Research Institute of Organic Agriculture (FiBL), Frick & IFOAM - Organics International, Bonn.

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Variability of non-starch polysaccharides, enzyme activity and rheological properties in rye (*Secale cereale* L.)

Christiane MAYR[1], Brigitte GRUBER[1, §], Pia ENGL[1], Heinrich GRAUSGRUBER[1]

[1] Department of Crop Sciences, University of Natural Resources and Life Sciences, Vienna, Konrad Lorenz-Str. 24, 3430 Tulln an der Donau, Austria

[§] present address: Seed Quality Testing, Corteva Agriscience Austria GmbH, Pioneerstraße Industriegelände, 7111 Parndorf, Austria

(✉) heinrich.grausgruber@boku.ac.at

Abstract

End-use quality of rye (*Secale cereale* L.) is largely dependent on the content of starch, the activity of α-amylase as the main starch degrading enzyme, and the concentration of pentosans. Starch and α-amylase activity are responsible for the crumb elasticity of the bread, whereas pentosans affect water absorption and dough viscosity and, therefore, bread volume (Weipert, 1998). Standard test procedures for the evaluation of rye quality are the falling number test according to Hagberg (HFN) and the determination of starch gelatinization properties predicted by the viscosity of a flour-water suspension during heating using the amylograph. High HFN and viscosity maxima in turn result in a very low enzyme activity of rye flours, making it necessary to add malted flour in the baking process to avoid 'dry baking' (Backaldrin, 2020). Recently, Oest *et al*. (2020) blamed also high amounts of insoluble non-starch polysaccharides (iNSPS) and a hampered denaturation of proteins to be responsible for rye bread defects. Within the RYE-SUS project two rye diversity panels were studied for quality traits: (i) nursery V31 included landraces and varieties from a breeding period of 142 year; (ii) nursery V32 included 96 near-isogenic experimental hybrids, half of them possessing the dominant dwarfing gene *Ddw1* (Kobyliansky, 1972).

Panel V31 consisted of 24 varieties (3 landraces, 17 populations, 4 hybrids) released between 1876 and 2018. The material was grown in 2020 and 2021 in Edelhof (48.607551 N, 15.214992 E) and Raasdorf (48.233154 N, 16.590265 E), Austria, at the latter site under both organic and conventional management. Panel V32 was tested only in 2021 in Edelhof and Raasdorf (organic). HFN was determined according to ICC Standard 107/1, rheological properties (viscosity) using a RapidVisco Analyzer (RVA 4500; PerkinElmer®). Contents of protein, starch, water extractable (WEAX) and total arabinoxylans (TAX) were determined by near-infrared-spectroscopy (NIRS) using the calibrations devised by Jürgens *et al*. (2012).

No influence of the test year was observed for HFN and RVA parameters, however, the effect of test site was significant with Edelhof (EHO) yielding inferior HFN and RVA values in both years (Fig. 1). HFN and viscosity was highly correlated (r = 0.94). A clear breeding progress in HFN and viscosity is visible for varieties released in the last two decades, especially the three German hybrid varieties outperformed the other test material (Fig. 2). Significant improvements to higher HFN and high amylogram maxima, especially in hybrid rye, were demonstrated also by Oberforster & Werteker (2011) and Laidig *et al*. (2017) for Austria and Germany, respectively. A significant year by test site interaction was observed for iNSPS (Fig. 3) with significant lower contents for the the two trials in Raasdorf in 2021. The iNSPS content was not correlated with HFN, RVA viscosity, WEAX and grain weight, but with grain size (r = 0.51) and TAX (r = 0.98). Hence, grain size seems to be an indicator for total and insoluble pentosan content, but not for the water soluble fraction. Kobylyansky *et al*. (2021) suggested to use the thickness of the seed coat as marker for a low water-soluble pentosan content with genotypes exhibiting a thin 'transparent' seed coat having lower levels.

In the V32 nursery with the experimental hybrids, the results gained by V31 could not be confirmed. In this trial no correlations were observed between grain size and TAX or iNSPS, but a weak one between grain size and WEAX (r = 0.33) (Fig. 4). Grain size was significantly smaller at both test sites in the hybrids carrying the *Ddw1* dwarfing gene. In almost all other quality parameters no significant differences were observed between the semi-dwarf and the tall hybrids. If significantly differences were revealed, these were due to the test environment. As for V31, this was especially the case for HFN and RVA parameters with lower values observed for samples from Edelhof. While the higher precipitation in Edelhof affected negatively HFN and viscosity it had a positive effect on grain size, thousand grain weight, test weight, and grain yield.

In summary, a clear relationship was found between HFN and viscosity parameters in both diversity panels. Contrary, the content of NSPS (*i.e*. total, soluble, insoluble) was not associated with rheological properties. Relationships between grain size and NSPS contents were not consistent in both panels. Hence, more detailed analyses considering the genetic background of the experimental hybrids is necessary if grain size is a stable indicator for any fraction of NSPS .

Mayr C, Gruber B, Engl P, Grausgruber H (2023) Variability of non-starch polysaccharides, enzyme activity and rheological properties in rye (*Secale cereale* L.). In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 61-63. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

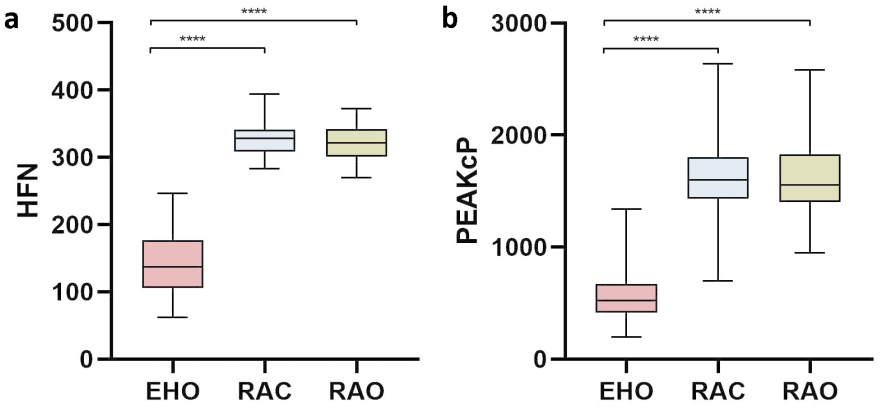

Figure 1 Variability of V31 in **a** falling number (HFN) and **b** viscosity (PEAKcP) in the different test environments (EHO, Edelhof conventional; RAC, Raasdorf conventional; RAO, Raasdorf organic). Data from 2020 and 2021 are merged due to no influence of the test year.

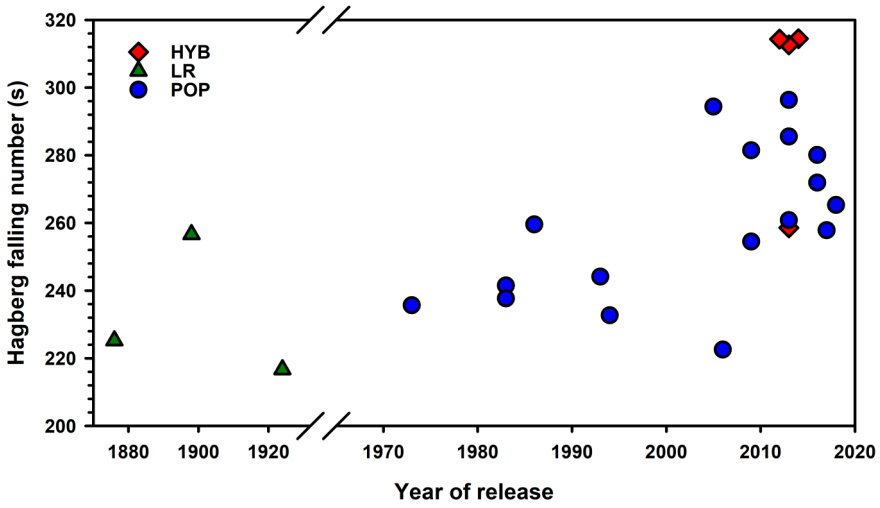

Figure 2 Breeding progress in Hagberg falling number: grand means plotted against the year of release of varieties (HYB, hybrid varieties; POP, OP population varieties; LR, landraces).

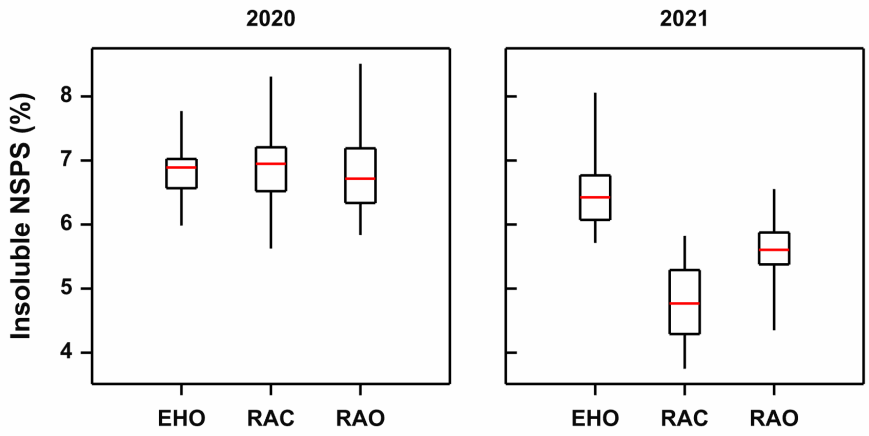

Figure 3 Year by test site interaction in the V31 diversity panel for the concentration of insoluble non-starch polysaccharides. EHO, Edelhof conventional; RAC, Raasdorf conventional; RAO, Raasdorf organic.

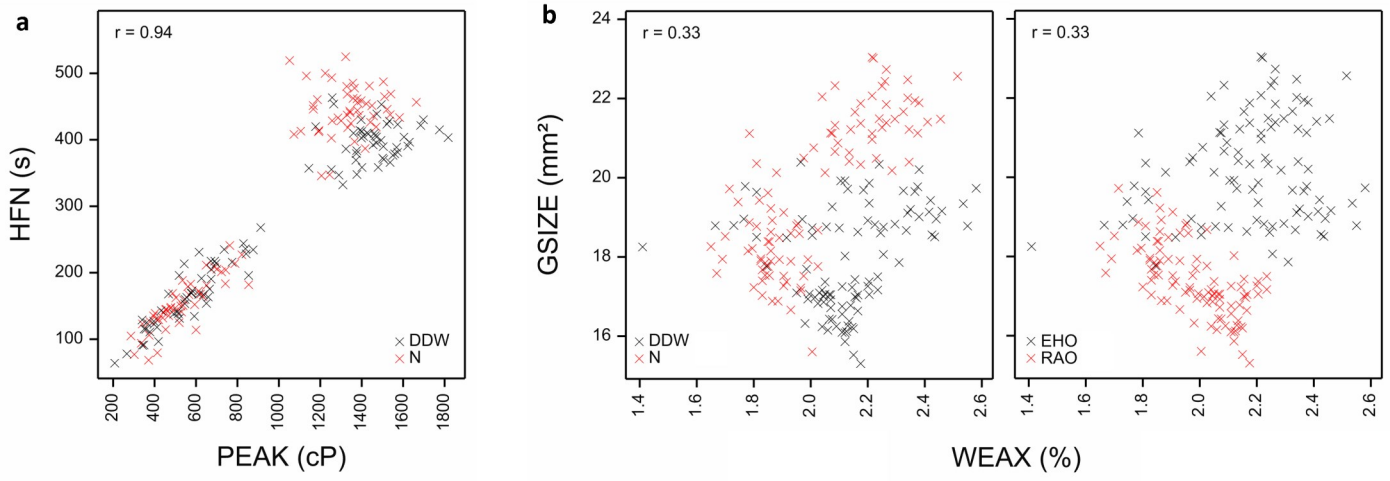

Figure 4 Relationships between **a** falling number (HFN) and viscosity (PEAK), and **b** grain size (GSIZE) and water-extractable arabinoxylans (WEAX) in the V32 nursery. Semi-dwarf (DDW) and tall (N) hybrids and test sites Edelhof conventional (EHO) and Raasdorf organic (RAO) are indicated by the respective keys. Clusters in graph **a** refer to the two test sites with EHO characterised by lower HFN and PEAK values.

Keywords

Arabinoxylan · baking quality · dwarfing gene · falling number · hybrid breeding · pentosans · *Secale cereale*

Acknowledgements

This research received funding by the Austrian Federal Ministry of Agriculture, Regions and Tourism (BMLRT; grant number 101407) within the ERA-Net Cofund Action under H2020 on Sustainable Crop Production (SusCrop; grant agreement 771134).

References

Backaldrin (2020) Deutscher Erntebericht 2020. Backaldrin, Asten.

Jürgens H-U, Jansen G, Wegener CB (2012) Characterisation of several rye cultivars with respect to arabinoxylans and extract viscosity. J Agric Sci 4(5):1-12. DOI: 10.5539/jas.v4n5p1

Kobyliansky VD (1972) On genetics of the dominant factor of short -strawed rye. Genetika 8:12-17 (in Russian, English summary).

Kobylyansky VD, Solodukhina OV, Nikonorova IM (2021) Morphological features of rye grain with low pentosan content. Proc Appl Bot Genet Breed 182:123-130 (in Russian, English abstract). DOI: 10.30901/2227-8834-2021-2-123-130

Laidig F, Piepho H-P, Rentel D, Drobek T, Meyer U, *et al*. (2017) Breeding progress, variation, and correlation of grain and quality traits in winter rye hybrid and population varieties and national on -farm progress in Germany over 26 years. Theor Appl Genet 130:981-998. DOI: 10.1007/s00122-017-2865-9

Oberforster M, Werteker M (2011) Inverse und nicht inverse Beziehungen von Kornertrag und Qualität im österreichischen Sortenspektrum von Weizen, Gerste und Roggen. 61. Jahrestagung 2010 der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs, pp 9-17. LFZ für Landwirtschaft, Raumberg-Gumpenstein, Irdning.

Oest M, Bindrich U, Voß A, Kaiser H, Rohn S (2020) Rye bread defects: Analysis of composition and further influence factors as determinants of dry-baking. Foods 9:1900: DOI: 10.3390/ foods9121900

Weipert D (1998) Roggen: Sorte und Umwelt. 2. Teil: Qualitätsmerkmale und Verarbeitungswert. Getreide, Mehl und Brot 52:259-263.

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Mycorrhization of rye (*Secale cereale* L.) cultivars under conventional and organic agriculture

Jordan LEBESMÜHLBACHER[1], Karin HAGE-AHMED[1], Heinrich GRAUSGRUBER[1]

[1] Department of Crop Sciences, University of Natural Resources and Life Sciences, Vienna, Konrad Lorenz-Str. 24, 3430 Tulln an der Donau, Austria

(✉) heinrich.grausgruber@boku.ac.at

Abstract

Arbuscular mycorrhizal fungi (AMF) are known for their symbiotic interaction with plants. The fungi grow into the cortex of the plant root and deliver nutrients from the soil through the hyphal network to the roots. Further, AMF can protect the plants from biotic and abiotic stress (Smith & Read, 2008). AMF also interact with different soil microorganisms and play a crucial role in the soil food web and nutrient circulation (Ordoñez *et al*., 2016). With their hyphal network and the excretion of sticky, recalcitrant substances like glomalin, AMF stabilize soil aggregates and support soil fertility in general (Agnihotri *et al*., 2022). Mycorrhizal symbiosis is widely distributed among plants and can be observed in approximately 80% of all plant species. However, the symbiotic interaction is dependent on several factors, such as climate, nutrient concentration in the soil, agricultural management and plant species or even cultivar (Smith & Read, 2008). While many crops have been investigated for their mycorrhizal interaction, data for rye (*Secale cereale*) are still rare. The aim of this work was to investigate the AMF colonization potential of different rye cultivars under organic and conventional farming.

The field trial was conducted in 2021 in Raasdorf on an organic and conventional site, being only 300 m away from each other. Soil type is a calcareous chernozem, soil texture varies from loam to loamy silt. The conventional field was fertilized with 160 kg N, 60 kg P and 90 kg K per hectare and treated with 1 L Axial® Komplett and 0.05 L Decis® Forte; precrop was durum wheat. At the organic site, no external fertilizer was added; the precrop was alfalfa. Six cultivars were tested, 3 landraces (*i.e*. 'Sangaste', 'Norddeutscher Champagnerroggen' and 'Lungauer Tauern-roggen'), two modern OP cultivars (*i.e*. 'Elego' and 'Elias') and one hybrid cultivar (*i.e*. 'SU Forsetti'). Sampling of plant material took place early June at heading (BBCH 55). For each plot, the roots of 4 plants were collected. The roots were washed and dyed with an ink-vinegar solution according to Vierheilig *et al*. (1998). Afterwards, the roots were evaluated under a microscope according to Trouvelot *et al*. (1986) (Fig. 1), using the Inoq-Calculator (Mercy, 2017) as support. Statistical analysis was caried out with R 4.0.0 (R Core Team) using a linear mixed model.

The results did not reveal significant differences between cultivars regarding mycorrhizal parameters. The landraces, OP cultivars and the hybrid cultivar showed a similar high frequency of AMF in the root system (Fig. 2A). The results show a snapshot of mycorrhizal root colonization at a specific developmental stage. The mycorrhizal symbiosis is a dynamic process and follows a sigmoidal curve, with slow colonization at the beginning, followed by rapid infection and a saturation at the end. Therefore, it can not be excluded, that differences in colonization might occur during different developmental stages. A significant and clear effect, however, was observed for the management system with a 11% higher frequency in average at the organic site (Fig. 2A). The intensity of AMF colonization was in average 16% for the conventional and 35% for the organic site. The intensity of colonization indicates the degree of colonization in the roots. Beside a higher frequency and intensity, plants from the organic site had a higher abundance of arbuscules, which was in average 18% higher (Fig. 2C). Arbuscules are mycorrhizal structures responsible for nutrient exchange between fungus and plant. Therefore, the abundance of arbuscules is an indicator for the functionality of symbiosis. Hence, it can be assumed that mycorrhizal symbiosis played a higher role in nutrient exchange at the organic site. The differences in the abundance of vesicles was not significant (Fig. 2D). Vesicles are bubble like structures of AMF responsible for nutrient storage. A high abundance of vesicles can indicate that AMF derive more nutrients from the plant, otherwise vesicles are important for fungal spread and colonization via AMF infected root fragments (Smith & Read, 2008). The effect of farming system on AMF has been reported also by other researchers. The use of synthetic fertilizers and pesticides affects mycorrhizal colonization. With high nutrient availability plants rather take up nutrients directly through roots, rather then investing energy in symbiotic interactions (Smith & Read 2008). Pesticides may have both positive and negative effects on AMF. Deltamethrin, the active compound of Decis® which was used on the conventional site, has been reported to negatively affect mycorrhizal colonization (Rivera-Becerril *et al*., 2017)

Keywords

Arbuscular mycorrhizal fungi · organic agriculture · plant-microbe interaction · roots · *Secale cereale* · soil microbiome · symbiosis

Lebesmühlbacher J, Hage-Ahmed K, Grausgruber H (2023) Mycorrhization of rye cultivars under conventional and organic agriculture. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 65-66. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

Acknowledgements

This research received funding by the Austrian Federal Ministry of Agriculture, Regions and Tourism (BMLRT; grant number 101407) within the ERA-Net Cofund Action under H2020 on Sustainable Crop Production (SusCrop; grant agreement 771134).

References

Agnihotri R, Sharma MP, Prakash A, Ramesh A, Bhattacharjya S, *et al*. (2022) Glycoproteins of arbuscular mycorrhiza for soil carbon sequestration: Review of mechanisms and controls. Sci Total Env 806:150571. DOI: 10.1016/j.scitotenv.2021.150571

Mercy L (2017) INOQ Calculator Advanced. Evaluate the mycorrhizal rate according to a modified Trouvelot method. DOI: 10.13140/RG.2.2.13641.03684

Ordoñez YM, Fernandez BR, Lara LS, Rodriguez A, Uribe-Vélez D, *et al*. (2016) Bacteria with phosphate solubilizing capacity alter mycorrhizal fungal growth both inside and outside the root and in the presence of native microbial communities. PloS One 11:e0154438. DOI: 10.1371/journal.pone.0154438

Rivera-Becerril F, van Tuinen D, Chatagnier O, Rouard N, Béguet J, *et al*. (2017) Impact of a pesticide cocktail (fenhexamid, folpel, deltamethrin) on the abundance of Glomeromycota in two agricultural soils. Sci Total Env 577:84-93. DOI: 10.1016/j.scitotenv.2016.10.098

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd ed. Elsevier, Amsterdam. DOI: 10.1016/B978-0-12-370526-6.X5001-6

Trouvelot A, Kough JL, Gianinazzi-Pearson V (1986) Mesure du taux de mycorhization VA d´un systéme radiculaire. Recherche de méthodes d´estimation ayant une signification fonctionnelle. In: Gianinazzi-Pearson V, Gianinazzi S (eds), Physiological and genetical aspects of mycorrhizae, pp 217-221. INRA, Paris.

Vierheilig H, Coughlan AP, Wyss U, Piché Y (1998) Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Appl Environ Microbiol 64:5004-5007. DOI: 10.1128/AEM.64.12.5004-5007.1998

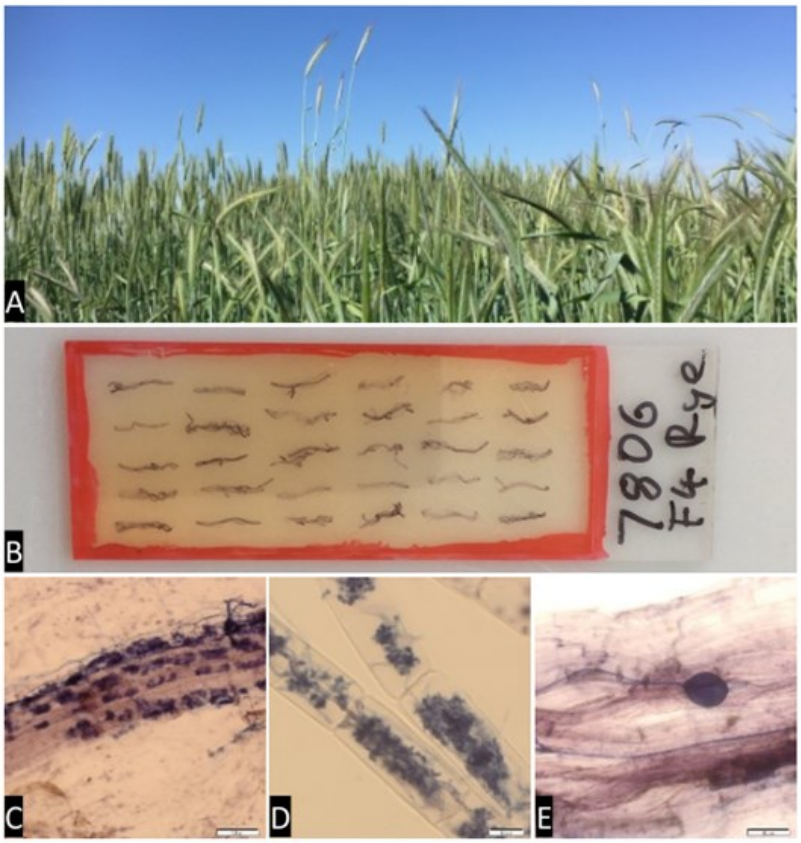

Figure 1 Mycorrhiza in rye: **a** rye plants at sampling (BBCH55); **b** microscope slide with 30 root fragments; **c** root fragment colonized by AMF; **d** close-up view of AMF-arbuscules; **e** close-up view of AMF-vesicles.

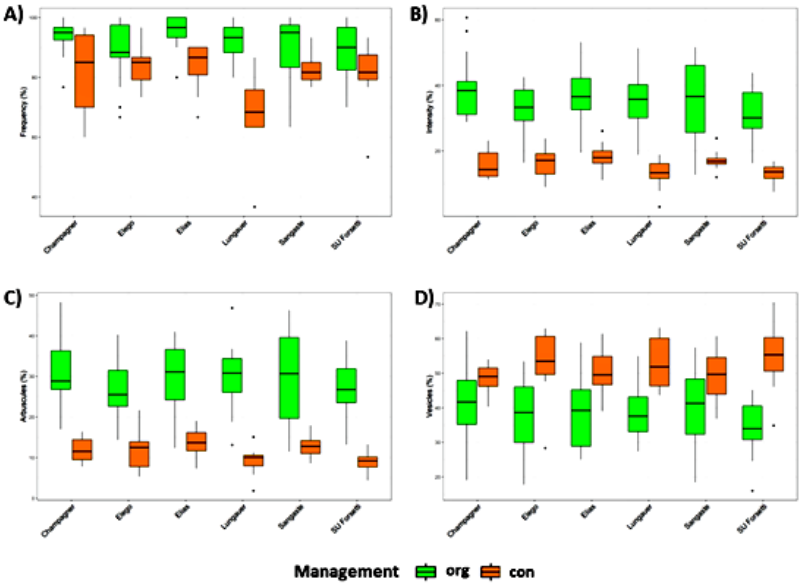

Figure 2 Variability of different mycorrhizal parameters: **a** frequency of mycorrhiza in the root system; **b** intensity of the mycorrhizal colonization; **c** abundance of arbuscules; **d** abundance of vesicles in the root system. Differences between organic and conventional management system were significant at $p < 0.01$ with the exception of **d**.

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Austrian potato variety trials in the ADAPT project

Alexandra RIBARITS[1], Svenja BOMERS[1], Susanne KIRCHMAIR[2], Philipp VON GEHREN[1], Josef SÖLLINGER[3]

[1] Austrian Agency for Health and Food Safety (AGES), Spargelfeldstraße 191, 1220 Vienna, Austria

[2] NÖ. Saatbaugenossenschaft (NÖS), Meires 25, 3841 Windigsteig, Austria

[3] Austrian Agency for Health and Food Safety (AGES), Wieningerstraße 8, 4020 Linz, Austria

(✉) alexandra.ribarits@ages.at

Abstract

The Horizon 2020 project ADAPT (Accelerated development of multiple-stress tolerant potato) investigates the molecular and phenotypic responses of potato (*Solanum tuberosum* L.) to abiotic stresses. The goal of ADAPT is to develop new strategies to make potatoes fit for the challenging climatic conditions of the future. The findings should facilitate the work of plant breeders in order to provide farmers with stress-tolerant potato varieties more quickly and efficiently. ADAPT combines the academic and applied expertise of 17 international project partners: Seven universities and three research institutions, four potato breeders, a screening technology developer, the European Potato Trade Association (Europatat) and AGES. Eight distinct work packages combine methods in molecular biology, stress physiology, systems biology and analytics with molecular breeding and sophisticated engineering. Work package 6 ("Pathways to impact") ensures the targeted and efficient exploitation and implementation of the project results, and their communication and dissemination to different target audiences. AGES is conducting field trials together with the Austrian potato breeding company NÖS to validate results from other work packages and to propose improvements to the standard value for cultivation and use (VCU) testing protocols.

In 2022, the first year of the Austrian potato variety trials was successfully accomplished. A set of 16 potato varieties was grown in five trials at four different locations in Austria. The 16 varieties were selected with a focus on representing a wide range of abiotic stress tolerance – from susceptible to tolerant. Varieties were obtained from the potato breeders involved in ADAPT: 'Acoustic', 'Lady Rosetta' and 'Musica' from Meijer Potato, 'Colomba' and 'Rosi' from HZPC, 'Belmonda' from Solana, and 'Erika', 'Valdivia', 'Chiara', 'Meireska', 'Ditta' and 'Graziosa' from NÖS. In addition, 'Agata', 'Agria', 'Désirée' and 'Spunta' were tested. The variety 'Désirée' serves as a check variety that is included in all trials in the ADAPT project. To reflect the importance of the organic sector in both Austria and the EU, the field trials were partially performed under organic farming conditions. All sites were located in Lower Austria, more specifically in Fuchsenbigl (48.19197, 16.74646) and Großnondorf (48.63361, 15.98386; both conventional), and in Sierndorf (48.426867, 16.167676) and Schwarzenau (48.743135, 15.259316; both organic). The standard protocol for VCU testing of potato in Austria was followed at all trial sites. The phenotypic evaluation of the varieties was complemented by different technological approaches to identify relevant characteristics associated with the responses of potato to abiotic stress factors: drone flights, environmental sensors, evaluation of tuberization as well as RNA and metabolomic analyses. From data collected by drone flights during two days in June and July, several indices that potentially give insights on how the plants cope with abiotic stresses were obtained, *i.e*. NDVI (Normalized Difference Vegetation Index), WDVI (Weighted Difference Vegetation Index), and CIRED (Chlorophyll Index based on NIR and RED band). In addition, vegetation coverage was determined to investigate the development of the varieties. Environmental data were collected by environmental sensors, including soil sensors. Air and soil temperature, air humidity and soil moisture were measured every 20 minutes over the course of the growing season. Onset of tuberization can have a significant impact on the yield potential of potato varieties. Therefore, additional plots were established at two trial sites (Fuchsenbigl and Großnondorf) to evaluate differences in the tuberization process among the 16 varieties at three time points in June and July. To observe the different stress responses of the varieties, one trial in Fuchsenbigl was irrigated four times during the growing season while the other received no irrigation. During two days in June and July, leaves were sampled for laboratory analyses to investigate stress-induced changes in physiology (metabolomics) and in gene expression (RNA).

The field trials generate large amounts of data that will help to unravel key components associated with adaptation to environmental stresses. The data will feed into the modeling approach by the ADAPT partner National Institute of Biology (NIB) in Slovenia (work package 5). We will furthermore assess the possibility of including the evaluation of abiotic stress tolerance as a characteristic in variety testing. In 2023, the same trials are planned to validate the results of 2022. Regular updates and more information can be found on the project's website (adapt.univie.ac.at) and Twitter account (@eu_Adapt).

Ribarits A, Bomers S, Kirchmair S, von Gehren P, Söllinger J (2023) Austrian potato variety trials in the ADAPT project. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 67-68. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/7875597

Figure 1 Different technological approaches to identify relevant characteristics associated with responses of potato to abiotic stress factors in the field trials in 2022: **a** drone flights; **b** environmental sensors; **c** evaluation of tuberization; **d** sprinkler irrigation; **e** leaf sampling for RNA and metabolomic analyses.

Keywords

Abiotic stress · climate change · drone · *Solanum tuberosum* · variety testing

Acknowledgements

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

Further reading

George TS, Taylor MA, Dodd IC, White PJ (2022) Climate change and consequences for potato production: a review of tolerance to emerging abiotic stress. Potato Res 60:239-268. DOI: 10.1007/s11540-018-9366-3

Handayani T, Gilani SA, Watanabe KN (2019) Climatic changes and potatoes: How can we cope with the abiotic stresses? Breed Sci 69:545-563. DOI: 10.1270/jsbbs.19070

Mulugeta Aneley G, Haas M, Köhl K (2023) LIDAR-based phenotyping for drought response and drought tolerance in potato. Potato Res, in press. DOI: 10.1007/s11540-022-09567-8

Obidiegwu JE, Bryan GJ, Jones HG, Prashar A (2015) Coping with drought: stress and adaptive responses in potato and perspectives for improvement. Front Plant Sci 6:542. DOI: 10.3389/fpls.2015.00542

Prashar A, Yildiz J, McNicol JW, Bryan GJ, Jones HG (2013) Infrared thermography for high throughput field phenotyping in *Solanum tuberosum*. PLoS One 8:e65816. DOI: 10.1371/journal.pone.0065816

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Pests and fungal diseases – how to save legume seed quality in times of climate change

Johann HUBER[1], Nicole CHALUPPA[1], Benno VOIT[2], Berta KILLERMANN[1]

[1] Bavarian State Research Center for Agriculture (LfL), Institute for Crop Science and Plant Breeding, Seed Research, Lange Point 6, 85354 Freising, Germany

[2] Ergolding, Landshut, Germany

(✉) johann.huber@lfl.bayern.de

Abstract

Bruchus rufimanus Boh. is a univoltine pest infesting faba beans (*Vicia faba* L.) during flowering and pod development. Due to climate change and the lack of natural insect antagonists in many areas, this bruchid species is meanwhile ubiquitous in all European countries and tempered regions worldwide. Bruchid infestation causes severe damage on germination and thus on seed quality. Although the germ buds in the seeds are very rarely impaired by the bruchid infestation, a higher percentage of abnormal seedlings is caused by faster seed imbibition and most notably the highly increased susceptibility to fungal pathogens. Consequently, the impairment of bruchid infestation on legume seed germination is depending on climate conditions during the vegetation period and the fungal disease occurrence at the seed production side.

From 2019 to 2021, three field trials each were conducted in organic and conventional cropping systems in three climatic and geographic different regions in southern, central and northern Germany to test various control strategies against the broad bean beetle. In order to distinguish between damage caused by alive bruchids in the seed beans and bruchids colonizing the fields during flowering, plot housings were installed over plots sown with beetle-free or infested seeds to prevent bruchid immigration and emigration.

Because these field trials did not provide consistent satisfying results for bruchid pest control, a new field trial series started in 2022 following a novel approach targeting the critical phase of the life circle of *B. rufimanus*: field colonisation before mating. To impair bruchid host plant location, a repellent was developed based on faba bean flower scent to shift the ratios between the crucial semio-chemical components necessary for host plant location. Because of natural behaviour patterns of *B. rufimanus* and because faba bean field are impassable at the end of the flowering period, common spray application and alternative application methods were tested, and a spray solution of rosemary essential oil was added as a natural reference. To develop a full-scale push-pull-strategy against the bruchids, advanced scent traps were additionally tested as the pull-component for mass trapping or separately to infect the caught bruchids with the entomopathogenic fungus *Beauveria bassiana*.

Two field trials were set up in a conventional faba bean field with a seed row spacing of 12.5 cm and an organic field with a seed row spacing of 50 cm, where weeding was conducted mechanically. The field trials were designed as four times replicated split plots with plots of 7 × 7 m and the repellent distributions as the whole plot and mass trapping (yes/no) as the subplot factors. Because of the high heterogeneity of bruchid infestation pressure within the faba bean fields, each replication was located in field border areas exposed to the estimated bruchid colonization direction and surrounded by untreated plot rows to ensure a small-scale detection of bruchid infestation and treatment effects.

In the 2019 to 2021 field trial series, late sowing (between 10th and 15th of May) was the only strategy that provided a significant reduction of bruchid infestation in all years and test sides. However, due to the negative impact on yield and TSW under dry conditions caused by the shortened vegetation period, late sowing is not suitable for bruchid control for most circumstances. Bruchids colonizing the field during flowering have a highly significant impact both on germination and on bruchid infestation, while alive bruchids in the seed beans have a subdominant role and only cause significant damage when the infestation pressure is remarkable low due to cold and wet conditions, as it was the case in 2021 (Table 1). In this context, the current legal advices for legume seeds containing alive bruchids in many European countries has to be questioned and new regulations should be discussed.

Keywords

Broad bean beetle · faba beans · push-pull-strategy · repellents · scent traps

Huber J, Chaluppa N, Voit B, Killermann B (2023) Pests and fungal diseases – how to save legume seed quality in times of climate change. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 69-70. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

Table 1 Impact of bruchids overwintering in outdoor habitats vs. in stored seed beans on germination and bruchid infestation from 2019 to 2021 in total and for each year separately. Significance levels: n.s., not significant, p >0.05; *, p <0.05; **, p <0.01; ***, p <0.001

Year	Bruchid overwintering strategy	Germination		Bruchid Infestation	
2019-2021	immigrating bruchids	$5.58 \cdot 10^{-6}$	***	$2.2*10^{-16}$	***
	alive bruchids in beans	0.207	n.s.	0.684	n.s.
2019	immigrating bruchids	$2.2 \cdot 10^{-16}$	***	$2.2*10^{-16}$	***
	alive bruchids in beans	0.206	n.s.	0.890	n.s.
2020	immigrating bruchids	0.022	*	0.027	*
	alive bruchids in beans	0.089	n.s.	0.601	n.s.
2021	immigrating bruchids	$5.95 \cdot 10^{-7}$	***	$2.2*10^{-16}$	***
	alive bruchids in beans	0.044	*	0.022	*

Acknowledgements

The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany through the Federal Office for Agriculture and Food (BLE), grant number 2818HS009, 2019 – 2023.

Further reading

Bruce TJ, Wadhams LJ, Woodcock CM (2005) Insect host location: a volatile situation. Trend Plant Sci 10:269-274. DOI: 10.1016/j.tplants.2005.04.003

Bruce TJ, Martin JL, Smart LE, Pickett JA (2011) Development of semiochemical attractants for monitoring bean seed beetle, *Bruchus rufimanus*. Pest Manag Sci 67:1303-1308. DOI: 10.1002/ps.2186

Sabbour MM, Abd El-Aziz SE, Shadia E (2007) Efficiency of some bioinsecticides against broad bean beetle, *Bruchus rufimanus* (Coleoptera: Bruchidae). Res J Agric Biol Sci 3:67-72.

Segers A, Megido RC, Lognay G, Francis F (2021) Overview of *Bruchus rufimanus* Boheman 1833 (Coleoptera: Chrysomelidae): Biology, chemical ecology and semiochemical opportunities in integrated pest management programs. Crop Prot 140:105411. DOI: 10.1016/j.cropro.2020.105411

Szafirowska A (2012) The role of cultivars and sowing date in control of broad bean weevil (*Bruchus rufimanus* Boh.) in organic cultivation. Vegetable Crop Res Bull 77:29-36. DOI: 10.2478/v10032-012-0013-2

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Towards heat tolerant runner bean (*Phaseolus coccineus* L.) by utilizing plant genetic resources

Svenja BOMERS[1], Eva M. MOLIN[2], Eveline ADAM[3], Philipp VON GEHREN[1], Karin HANSEL-HOHL[2], Noémie PRAT[1], Alexandra RIBARITS[1]

[1] Austrian Agency for Health and Food Safety (AGES), Division for Food Security, Institute for Seed and Propagating Material, Phytosanitary Service and Apiculture, Spargelfeldstr. 191, 1220 Vienna, Austria

[2] Austrian Institute of Technology (AIT) GmbH, Center for Health & Bioresources, Konrad-Lorenz-Str. 24, 3430 Tulln an der Donau, Austria

[3] Saatzucht Gleisdorf GesmbH, Am Tieberhof 33, 8200 Gleisdorf, Austria

(✉) alexandra.ribarits@ages.at

Abstract

Runner bean is a traditional product of the province of Styria where ≈90% of all runner beans in Austria are produced. The denomination "Steirische Käferbohne" was entered in the register of protected designations of origin (PDO) and protected geographical indications (PGI) in August 2016 and describes climbing runner bean varieties with purple-black to brown-beige speckled seeds, complying with the Austrian varieties 'Bonela' and 'Melange'. In recent years, during hot and dry summers, major crop losses have occurred in the production of runner bean in Styria. Especially the hot summers of 2003, 2013 and 2015 caused almost a total crop failure resulting in yields of only 297 kg ha^{-1}, 130 kg ha^{-1} and 139 kg ha^{-1}, respectively, which is only about a tenth of the usual yield potential in intercropping systems in Austria. As summers in Austria are hot and predicted to get even hotter in future, varieties with increased heat tolerance are required.

In our study, we assessed genetic and phenotypic characteristics of 113 runner bean accession grown in the greenhouse under heat stress conditions in two years (2018 & 2020). The material included 100 plant genetic resources (PGRs) obtained from the gene bank of AGES in Linz, 11 runner bean varieties including 'Bonela' and 'Melange', as well as 2 breeding lines. In 2018, 33 genotypes with 8 individuals each and in 2020, 64 genotypes with 6 individuals each were cultivated in pots in the greenhouse. Heat stress conditions with temperatures reaching more than 32°C were ensured either by heating (in 2018) or by natural conditions (in 2020). Honey bee colonies were placed in the greenhouses to ensure pollination. The number of flowers and the number of formed pods on each individual plant were counted three times a week during the flowering period and the number of beans was evaluated for each genotype after the harvest. For a majority of the genotypes (*i.e*. 94), DNA from eight biological replicates was sent to Floragenex, Beaverton, OR, for restriction site associated DNA sequencing (RADseq) in 2018. With the use of the MassARRAY® system in 2020, selected genotypes were genotyped by using selected SNPs (Fig. 1).

All genotypes reacted towards the heat stress with shedding of flowers and stop of flowering, resulting in a rapidly declining number of flowers. In 2018, the cumulative number of flowers of all individuals declined from a maximum of 1884 flowers to a minimum of 70 flowers after 17 days of continuous heat stress. After harvest, large differences in number and appearance of the beans between the genotypes were observed. In 2018, nine accessions and one variety developed more beans under heat stress conditions than 'Bonela'. In 2020, 19 accessions, one breeding line and three varieties developed more beans under heat stress conditions than 'Bonela'. In total, 24 genotypes were higher yielding under heat stress conditions than 'Bonela' in at least one experimental year. In particular, three PGRs from the Austrian gene bank showed a higher yield than 'Bonela' under heat stress in both experimental years: BVAL-610181 is a PGR from South-East Styria which flowers red and forms purple-black speckled beans; BVAL-610348 is a PGR from South Italy with white flowers and white beans; BVAL-610637 is a PGR from Burgenland with red flowers and purple-black speckled beans. The combination of the geno- and phenotype in the genome-wide association analysis (GWAS) resulted in 18 high quality SNPs that were subsequently used for the calculation of an estimated heat tolerance using the MassARRAY® system. In 2018 and 2020, the average heat tolerance across all phenotyped genotypes was 54%. Most of the genotypes with an above-average number of beans also had an above-average estimated heat tolerance. The reference variety 'Bonela' had an average estimated heat tolerance of 56%. The three promising PGRs presented above had an above-average estimated heat tolerance of 62%, 63% and 81%. In our analysis, genotypes with an estimated heat tolerance of >60% were likely to have higher yield under heat stress conditions (heat tolerant). Genotypes with an estimated heat tolerance of <50% were likely to develop fewer beans under heat stress conditions (not heat tolerant). From the 33 PGRs that have not yet been phenotypically evaluated in our study, 13 are potentially not heat tolerant (<50%) and seven are potentially heat tolerant (>60%). For agronomical reasons it will also be important to see whether these genotypes also perform well and produce high yields in field conditions, in particular in hot and dry summers.

Bomers S, Molin EM, Adam E, von Gehren P, Hansel-Hohl K, *et al*. (2023) Towards heat tolerant runner bean (*Phaseolus coccineus* L.) by utilizing plant genetic resources. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 71 -72. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

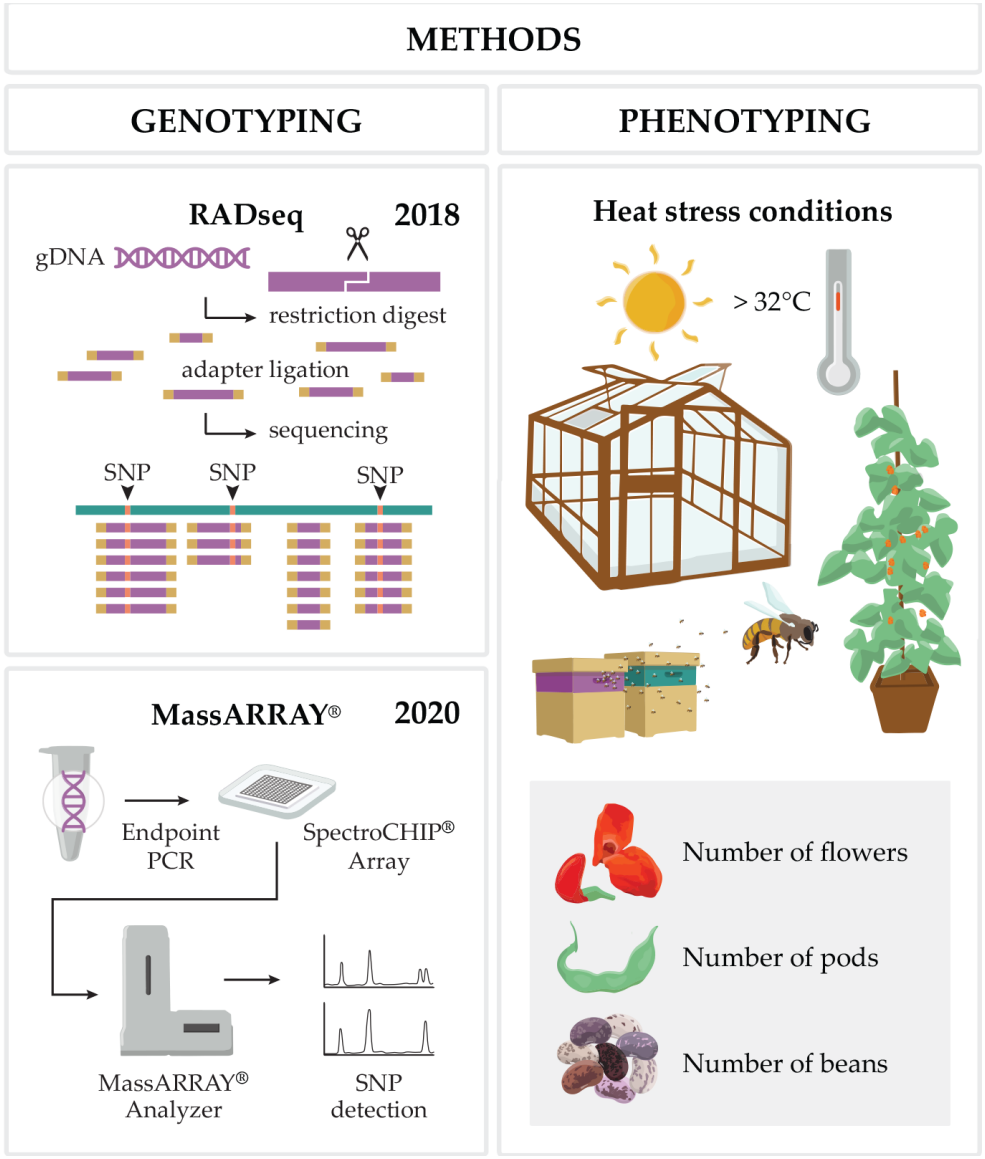

Figure 1 Methods used for genotyping, RADseq in 2018 and the MassARRAY® system in 2020, and the phenotyping under heat stress conditions.

Overall, our study represents first steps towards breeding heat tolerant runner bean varieties to adapt to a warming climate. The findings can speed up the development of new runner bean lines and make it less labor intensive and costly than it would be possible without using molecular markers. Not only can the promising heat-tolerant PGRs identified here be used as crossing partners, but also the 18 SNP markers can be used to identify and select heat-tolerant progeny after crossing trials.

Keywords

GWAS · heat stress · MassARRAY · plant genetic resources · RADseq

Acknowledgements

This research was funded by the Austrian Federal Ministry for Agriculture, Regions and Tourism and the Province of Styria within the framework of the federal/provincial cooperation on research (DaFNE-Research project 101193, "Genomics and phenomics of Austrian scarlet runner bean accessions with the focus on heat tolerance", https://dafne.at/projekte/characcess, and 101536, "Development of molecular markers to facilitate runner bean breeding", https://dafne.at/projekte/characcessii). The authors thank Tatjana Hirschmugl (https://scillustration.at/en/) for the graphic design.

Further reading

Bomers S, Sehr EM, Adam E, von Gehren P, Hansel-Hohl K, *et al*. (2022) Towards heat tolerant runner bean (*Phaseolus coccineus* L.) by utilizing plant genetic resources. Agronomy 12:612. DOI: 10.3390/agronomy12030612

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Infrared spectroscopy for rapid mycotoxin screening

Stephan FREITAG[1], Michael SULYOK[1], Andreas ZITEK[2,3], Heinrich GRAUSGRUBER[4], Rudolf KRSKA[1,2]

[1] University of Natural Resources and Life Sciences, Vienna, Department of Agrobiotechnology, IFA-Tulln, Institute of Bioanalytics and Agro-Metabolomics, Konrad-Lorenz-Str. 20, 3430 Tulln an der Donau, Austria

[2] FFoQSI GmbH - Austrian Competence Centre for Feed and Food Quality, Safety and Innovation, Technopark 1D, 3430 Tulln, Austria

[3] University of Natural Resources and Life Sciences, Vienna, Department of Chemistry, Muthgasse 18, 1190 Vienna, Austria

[4] University of Natural Resources and Life Sciences, Vienna, Department of Crop Sciences, Konrad-Lorenz-Str. 24, 3430 Tulln, Austria

(✉) stephan.freitag@boku.ac.at

Abstract

Mycotoxins (MT) are a diverse group of fungal metabolites hazardous to humans and animals (Alshannaq & Yu, 2017). A wide variety of analytical methods exists for their analysis, immunoanalytical techniques like enzyme-linked immunosorbent assays (ELISA) and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) being the most prominent ones. Even though these methods are the work horses for MT screening, they come with certain limitations. Both methodologies rely on laboratory equipment like mills, reagents, and consumables for sample preparation, hampering their on-site implementation. Infrared (IR) spectroscopy on the other hand requires minimal to no sample preparation, thereby facilitating high analytical throughput with simplified device operation. Near infrared spectroscopy (NIRS) has been used for the analysis of agricultural commodities for several decades and IR spectroscopy demonstrated great potential for MT screening (Freitag *et al*., 2022). However, the indirect nature of these methods, as MT contamination is linked with fungi induced sample changes using chemometric methods, makes method development challenging. Furthermore, reports on the usage of portable NIR spectrometers for MT screening are scarce. The aim of this study was to benchmark a handheld against a benchtop NIR spectrometer for the screening of deoxynivalenol (DON) in intact wheat validated by LC-MS/MS data.

We analyzed 194 winter and durum wheat samples for their contamination with DON including different varieties from different regions. DON levels were obtained by LC-MS/MS and later used as reference during multivariate analysis. The details of the LC-MS/MS method can be found in (Sulyok *et al*., 2020). Near IR (NIR) spectra of wheat kernels were recorded using a Foss DS 2500 (Foss, Hillerød, Denmark) benchtop spectrometer (400 - 2498 nm, 0.5 nm spectral resolution), as well as a handheld MicroNIR spectrometer (950 - 1650 nm, 7 nm spectral resolution, VIAVI Solutions, Scottsdale, Arizona). Spectra processing and multivariate analysis was done using the statical computing environment RStudio. The packages caret and prospectr were used for spectra pre-processing and partial least squares discriminant analysis (PLS-DA) (Kuhn, 2008; Stevens & Ramirez–Lopez, 2014). Second derivatives were chosen as spectra preprocessing method, with a widow size of 11 and a segment size of 10.

During LC-MS/MS analysis it was found that 22 out of the 194 samples were exceeding the EU limit of 1250 ppb for DON in wheat. Hereafter, these samples are referred to as high contaminated (hC) and the rest of the sample set (n = 172) as low contaminated (lC). This imbalance between hC and lC samples can be expected in naturally contaminated wheat. However, for building classification models such imbalances are challenging, ideally the samples are evenly distributed among the classes. Therefore, balancing the classes artificially during model building was explored. The sample set was randomly split into a training (80%) and test set (20%) during multivariate analysis. The training set contained 18 and the test set 4 hC samples. 10-fold 10-time repeated cross-validation was used during model building. Down sampling (DS) was done by randomly selecting lC to match the amount of hC in the training set.

Fig. 1 shows the classification performance of the different PLS-DA models on the test set. The 4 hC samples were correctly identified with the PLS model based on the benchtop data; however, 12 samples were falsely classified as lC (Fig. 1a). This is linked to the fact that during DS only few lC samples of the training set are selected, not representing the whole variance in the lC class to the PLS algorithm. When using data obtained with the portable device and DS a similar performance was found, but not all hC samples could be correctly identified (Fig. 1c), which might be linked to the smaller spectral range. When using the raw data set, without down sampling none of the hC samples could be identified using NIRS (Figs. 1b & 1d). This shows that similar performance regarding mycotoxin screening can be obtained using a portable spectrometer compared to the benchtop device. Re-sampling strategies during multivariate analysis enhance the performance of NIRS MT prediction methods. We demonstrated that exploratory model development for NIRS MT screening at

Freitag S, Sulyok M, Zitek A, Grausgruber H, Krska R (2023) Infrared spectroscopy for rapid mycotoxin screening. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 73-74. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

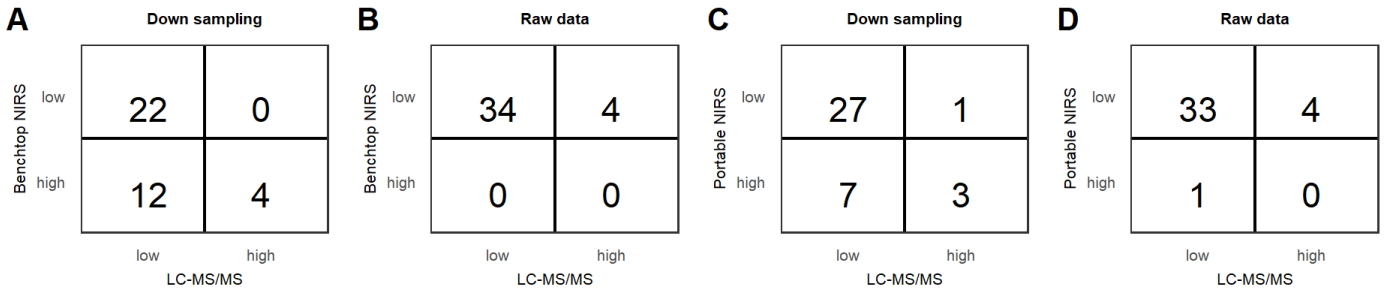

Figure 1 Classification results as confusion matrix of the actual class (LC-MS/MS) versus the predicted class (NIRS), for the benchtop device using **a** down sampling, **b** the raw imbalanced dataset, as well as the portable device using **c** down sampling, and **d** the raw data set.

regulatory limits is feasible, even with a non-ideal small sample set and a portable device, by using resampling methods during multivariate data analysis. Our subsequent research will focus on the extension of the data set to improve the model performance.

Keywords

Food safety · Fusarium · grain analysis · infrared spectroscopy · mycotoxin · *Triticum* · wheat ·

Acknowldedgements

This research is part of the PHOTONFOOD project, which received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 101016444 and is part of the PHOTONICS PUBLIC PRIVATE PARTNERSHIP. This work was also supported by a research project of the Austrian Competence Centre for Feed and Food Quality, Safety and Innovation (FFoQSI). The COMET-K1 competence centre FFoQSI is funded by the Austrian federal ministries BMK, BMDW and the Austrian provinces Lower Austria, Upper Austria and Vienna within the scope of COMET - Competence Centers for Excellent Technologies. The programme COMET is handled by the Austrian Research Promotion Agency FFG.

References and further reading

Alshannaq A, Yu J-H (2017) Occurrence, toxicity, and analysis of major mycotoxins in food. Int J Environ Res Public Health 14:632. DOI: 10.3390/ijerph14060632

Freitag S, Sulyok M, Logan N, Elliott CT, Krska R (2022) The potential and applicability of infrared spectroscopic methods for the rapid screening and routine analysis of mycotoxins in food crops. Compr Rev Food Sci Food Safety 21:5199-5224. DOI: 10.1111/1541-4337.13054

Kuhn M (2008) Building predictive models in R using the caret package. J Stat Software 28 (5):1-26. DOI: 10.18637/jss.v028.i05

Stevens A, Ramirez-Lopez L (2014) An introduction to the prospectr package. [https://antoinestevens.github.io/prospectr/; updated version see https://github.com/l-ramirez-lopez/prospectr; accessed 24 Jan 2023].

Sulyok M, Stadler D, Steiner D, Krska R (2020) Validation of an LC-MS/MS-based dilute-and-shoot approach for the quantification of >500 mycotoxins and other secondary metabolites in food crops: Challenges and solutions. Anal Bioanal Chem 412:2607. DOI: 10.1007/s00216-020-02489-9

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Estimation of canopy parameters in wheat using radiative transfer model inversion based on an artificial neural network

Lukas KOPPENSTEINER[1,§], Reinhard NEUGSCHWANDTNER[1], Sebastian RAUBITZEK[2], Philipp WEIHS[3], Norbert BARTA[4], Pablo RISCHBECK[1], Thomas NEUBAUER[2], Pia EUTENEUER[1], Andreas GRONAUER[4], Hans-Peter KAUL[1]

[1] Department of Crop Sciences, University of Natural Resources and Life Sciences, Vienna, Konrad-Lorenz-Str. 24, 3430 Tulln, Austria

[2] Institute of Information Systems Engineering, TU Wien, Favoritenstr. 11, 1040 Wien, Austria

[3] Department of Water, Atmosphere and Environment, University of Natural Resources and Life Sciences, Vienna, Gregor-Mendel-Str. 33, 1180 Wien, Austria

[4] Department of Sustainable Agricultural Systems, University of Natural Resources and Life Sciences, Vienna, Peter-Jordan-Str. 82, 1190 Wien, Austria

§ present address: Saatzucht Edelhof GmbH, Edelhof 4, 3910 Zwettl, Austria

(✉) lukas.koppensteiner@saatzucht-edelhof.at

Abstract

The objective of this contribution is to present preliminary results on the estimation of canopy parameters in wheat, such as leaf area index, chlorophyll content, equivalent water thickness and brown pigment content, based on hyperspectral reflectance measurements with a handheld spectroradiometer. To do so, the radiative transfer model PROSAIL was inverted and an artificial neural network was applied. The model was trained and tested using a simulated dataset. Results of the simulated dataset show that the inversion of PROSAIL based on an artificial neural network was successful. Furthermore, estimations of leaf area index compared to experimentally collected data on green area index feature high R^2 and low RRMSE. The technique proposed in this study is a promising tool to collect information on canopy characteristics of wheat in a quick and non-destructive way with low calibration requirements. This can be utilized by practical farmers for field monitoring as well as scientists and breeders for quick and non-destructive data collection in field experiments. Additionally, this approach can be adapted for different crops and varying sensors, *e.g*. multi- and hyperspectral UAV-mounted sensors as well as satellite data.

Keywords

Hyperspectral imaging · machine learning · PROSAIL · remote sensing · *Triticum aestivum*

Introduction

Remote sensing allows quick and non-destructive measurements of canopy characteristics. Commonly, vegetation indices are applied, however, this approach usually requires continuous calibration and cannot use all available spectral data for analysis. Radiative transfer models (RTMs) are a promising alternative to vegetation indices. These models describe the interaction between solar radiation and vegetation canopy (Monteith, 1965). Compared to vegetation indices, RTMs generalize well, have low calibration needs and allow analysis of all available spectral data (Berger *et al*., 2018).

Material and methods

The RTM PROSAIL simulates the spectral reflectance of vegetation canopy from 400 to 2500 nm in 1 nm increments using information on leaf characteristics, canopy architecture, viewing geometry and other effects (Fig. 1). Simulations in the RTM PROSAIL (vers. 5B) were conducted using the package hsdar (vers. 1.0.3) in R software (vers. 4.1.1) (Lehnert *et al*., 2019).

A simulated dataset consisting of 100,000 observations was created for model training and testing. Each observation included a random set of PROSAIL input parameters drawn from uniform distributions of the PROSAIL input parameters within wheat-specific ranges from literature (Kong *et al*., 2016; Danner *et al*., 2017). Furthermore, spectral reflectance for background soil was varied among observations in the simulated dataset. To do so, available data on soil reflectance by the ICRAF-ISRIC Soil MIR Spectral Library of the International Soil Reference and

Koppensteiner L, Neugschwandtner R, Raubitzek S, Weihs P, Barta N, *et al*. (2023) Estimation of canopy parameters in wheat using radiative transfer model inversion based on an artificial neural network. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 75-78. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

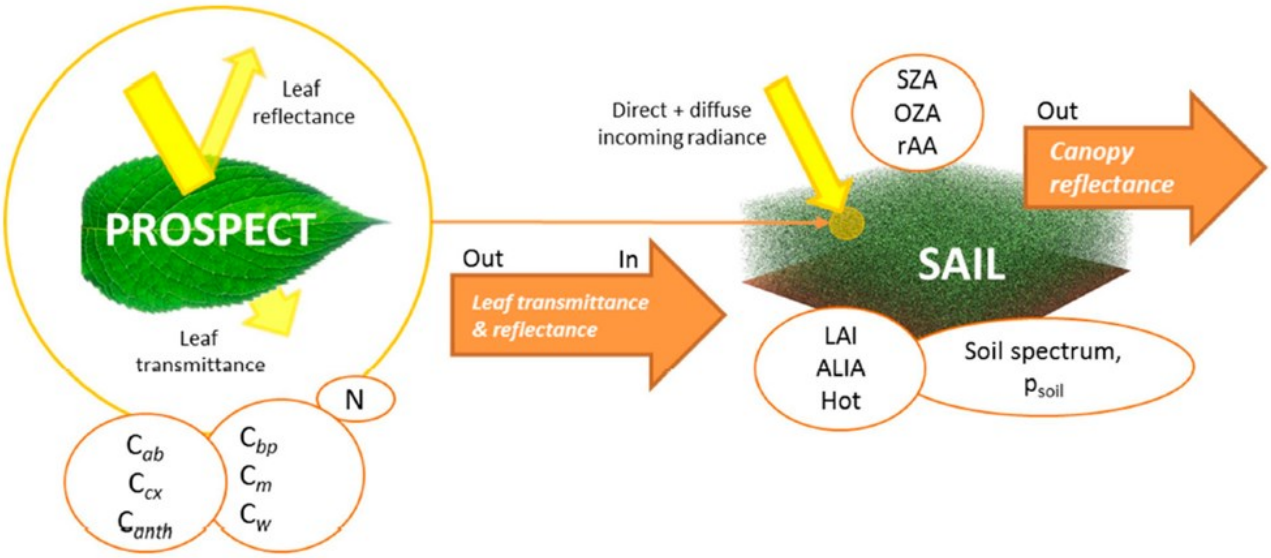

Figure 1 Calculation of canopy reflectance using the coupled PROSPECT + SAIL model (PROSAIL). N, leaf structure index; C_{ab}, chlorophyll a + b content (µg cm^{-2}); C_{cx}, carotenoid content (µg cm^{-2}); C_{anth}, anthocyanin content (µg cm^{-2}); C_{bp}, brown pigment content; C_m, dry matter content (g cm^{-2}); C_w or EWT, equivalent water thickness (mL cm^{-2}); LAI, leaf area index (m^2 m^{-2}); ALIA, average leaf inclination angle (°); Hot, hot-spot parameter (m m^{-1}); soil spectrum (% reflectance); psoil, soil brightness factor; SZA, sun zenith angle (°); OZA, observer zenith angle (°); rAA, relative azimuth angle (°).

Information Centre (ISRIC) were used (van Reeuwjik, 2002). The simulated dataset was divided into a train and test set in a 9:1 ratio.

Field experiments were conducted at the Experimental Farm Groß-Enzersdorf of the University of Natural Resources and Life Sciences, Vienna, in the seasons 2019/20 and 2020/21. Data on canopy parameters, such as green area index (GAI, m^2 m^{-2}), were collected in approximately 14-day intervals from March until harvest in July in both seasons. Measurements on canopy reflectance in the field experiment were conducted with the spectroradiometer ASD FieldSpec® Handheld 2 (Malvern Panalytical Ltd., Malvern, UK). This sensor provides hyperspectral reflectance data from 325 to 1075 nm in 1 nm increments.

An artificial neural network (ANN) was set up to achieve the inversion of the radiative transfer model PROSAIL. Model inputs were viewing geometry, background soil reflectance and canopy reflectance from 400 to 1075 nm in 1 nm increments. The spectral resolutions of soil reflectance, simulated PROSAIL canopy reflectance and spectral measurements from the field experiments were matched. Model outputs were the PROSAIL parameters N, C_{ab}, C_{cx}, C_{bp}, C_m, EWT, LAI, ALIA and Hot. The ANN consisted of three dense layers with 128 neurons each, ReLU activation function, loss function "mean absolute error" and optimizer "Adam". Training epochs were set to a maximum of 500 with early stopping at 50 to avoid overfitting. Google Colaboratory, an available Keras implementation (vers. 2.8.0) in Python (vers. 3.6; Python Software Foundation, Beaverton, USA), was used to set up the ANN. Experimentally measured GAI was estimated using predicted LAI.

The accuracy of model predictions compared to measured values was evaluated using regression coefficients and coefficients of determination (R^2) in regression analysis. Furthermore, root mean square error (RMSE) and relative root mean square error (RRMSE) were calculated for model testing.

Results and discussion

Fig. 2 presents the results on the comparison between true and predicted canopy parameter values in the simulated test dataset. The parameters LAI, C_{ab}, EWT and C_{bp} show very high R^2, *i.e*. above 0.9. This indicates, that the ANN based inversion of the RTM PROSAIL was successful. Furthermore, C_{ab}, EWT and C_{bp} indicate linear relationship between true and predicted values, while LAI features a quadratic relationship. When no leaf area is present (LAI = 0 m^2 m^{-2}), the parameters C_{ab}, EWT and C_{bp} could not be estimated. For observations with low LAI, *e.g*. below 0.5 m^2 m^{-2}, predictions on C_{ab}, EWT and C_{bp} showed systematic deviations. This can be explained by the high influence of background soil reflectance on spectral measurements, when LAI is low.

Model predictions on LAI were calibrated using experimental data on GAI from 2020/21. The calibrated predictions of GAI were validated using experimental data from 2019/20 (Fig. 3). In both seasons, R^2 values were high, *i.e*. above 0.8. In the experimental validation data of 2019/20, the deviation from the 45° line was low. These results indicate a high predictability of GAI based on our model as well as high stability among seasons.

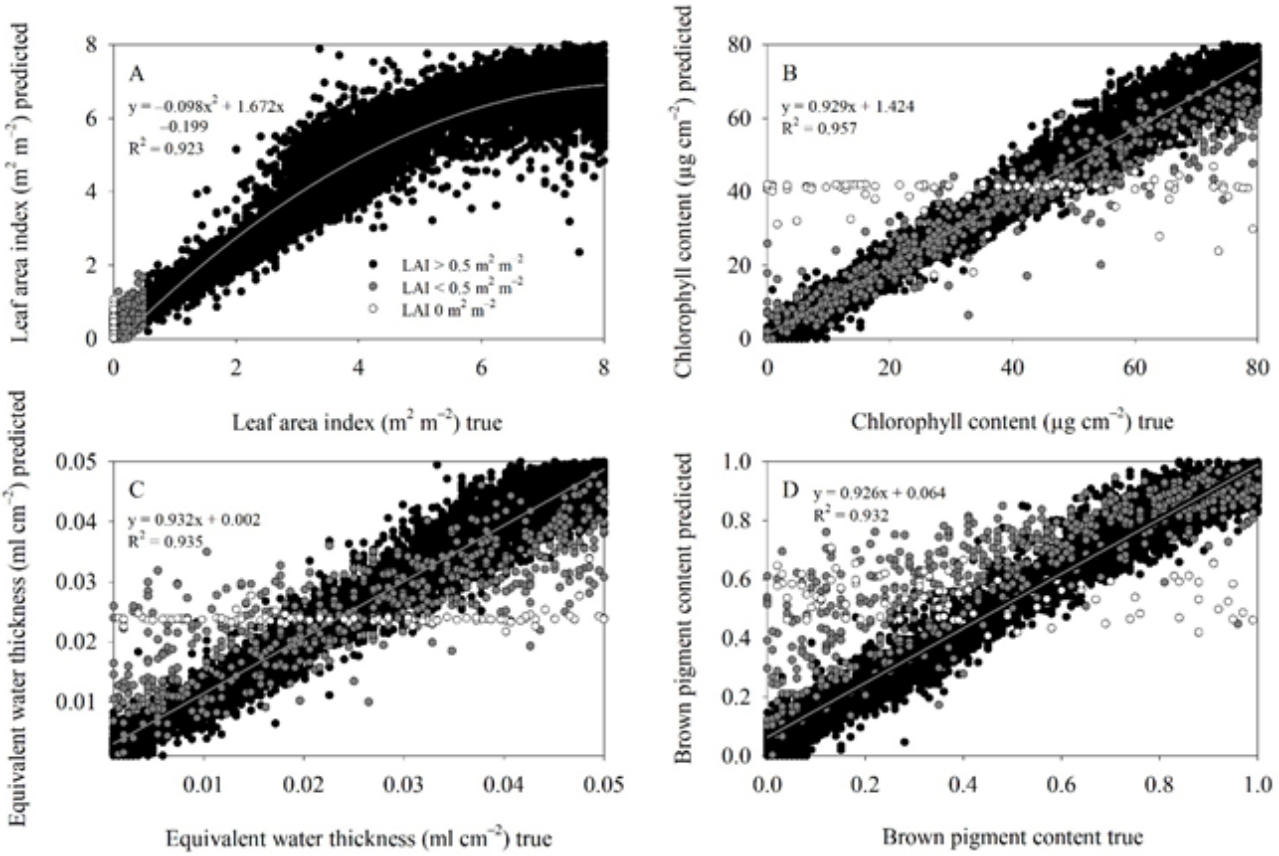

Figure 2 Estimation of **a** leaf area index (A, m^2 m^{-2}), **b** chlorophyll content (B, µg cm^{-2}), **c** equivalent water thickness (C, mL cm^{-2}) and **d** brown pigment content (D) of the simulated test dataset.

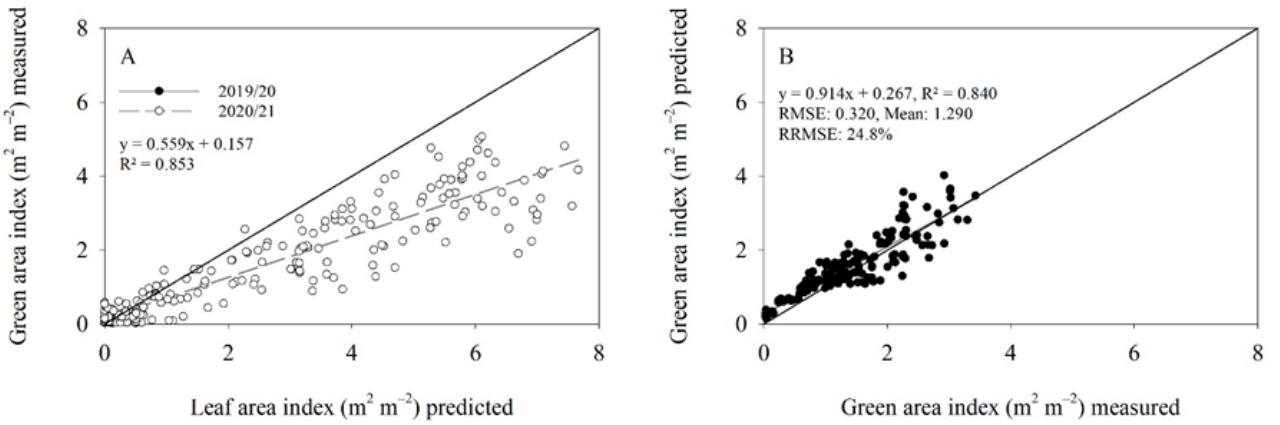

Figure 3 Calibration of **a** predicted leaf area index (m^2 m^{-2}) with measured green area index (m^2 m^{-2}) of the field experiment in 2020/21 as well as **b** validation of the calibrated predictions on green area index with respective measurements of the field experiment in 2019/20 .

Acknowledgements

This work was supported by the project "DiLaAg – Digitalization and Innovation Laboratory in Agricultural Sciences" of the private foundation "Forum Morgen, Austria" and the Federal State of Lower Austria.

References

Berger K, Atzberger C, Danner M, D'Urso G, Mauser W, *et al.* (2018) Evaluation of the PROSAIL model capabilities for future hyperspectral model environments: a review study. Remote Sens 10:85-110. DOI: 10.3390/rs10010085

Danner M, Berger K, Wocher M, Mauser W, Hank T (2017) Retrieval of biophysical crop variables from multi-angular canopy spectroscopy. Remote Sens 9:726. DOI: 10.3390/rs9070726

Kong WP, Huang WJ, Zhou XF, Song XY, Casa R (2016) Estimation of carotenoid content at the canopy scale using the carotenoid triangle ratio index from in situ and simulated hyperspectral data. J Appl Remote Sens 10:026035. DOI: 10.1117/1.JRS.10.026035

Lehnert LW, Meyer H, Obermeier WA, Silva B, Regeling B, *et al*. (2019) Hyperspectral data analysis in R: The hsdar package. J Stat Software 89(12):1-23. DOI: 10.18637/jss.v89.i12

Monteith JL (1965) Light distribution and photosynthesis in field crops. Ann Bot 29:17-37. DOI: 10.1093/oxfordjournals.aob.a083934

van Reeuwijk LP (2002) Procedures for soil analysis, 6th ed. International Soil Reference Information Centre, Wageningen.

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Digital solutions in plant breeding

Svenja DIEHL[1], Maximilian MAYER[1], Matti WAACK[1], Rico BASEKOW[1]

[1] Wintersteiger AG, Dimmelstr. 9, 4910 Ried im Innkreis, Austria

(✉) svenja.diehl@wintersteiger.com

Abstract

'There is no alternative to digital transformation' (Jeff Bezos)

In this digital world, paper atlases and road maps are no longer used when we go on a trip. Even the most outlying breeding station can be navigated via smartphone, and in most cases, we arrive at our destination at the estimated arrival time. Money transfers via online banking, music and movies via streaming, shopping without leaving the house. Digitized processes have become an integral part of our everyday lives. They mean convenience and completely new opportunities, but also challenges.

In plant breeding and field trials, digitization is in full swing, yet still in its infancy. Combines have become small laboratories with automated weighing systems and other analytical devices that collect plot-by-plot data. Drones and robots provide us with digital images and weather stations, and soil sensors generate more data. Full-genome chips give us a glimpse of the genetic make-up of our variety of the future. Many other promising technologies are under way. So far so good - a lot of data, a lot of digitization, and yet nothing is gained until all the data flows together correctly. Due to the lack of standardized interfaces, this is still difficult. The all-important uniform integration of data currently requires individual solutions and often still the manual input that we intended to save with digitization.

The 'right' data basis is also the key in trials. From planning the trials to deciding on the best variety, fertilizer or pesticide, many individual steps follow. The output from one step provides the input for the next. If the data is available in standard digital formats, the process steps can be combined into an automated workflow. This is the only way to save time, reduce costs, increase transparency, and avoid errors. At Wintersteiger, we take exactly this approach. Various digitized process steps are interlinked. Collected data is integrated automatically and is available for the next step. Our Easy Breed software (easybreed.com) plays a central role in this process. The software consists of a database, a user interface, and an interface to analysis tools.

Trial design and error-free seeding is the necessary basis for the later evaluation of the collected data and thus the decision on the best variety. A major advance is the GIS-based trial planning and later seeding. Seeding triggered by GPS signals saves time and avoids mistakes.

Easy Breed, MiniGIS, Easy Plant, as well as the TopCon guidance system and the Dynamic Disc Plus and Plot Motion seed drills are coordinated for GIS-based seeding so that all individual steps run in a coordinated manner. The complete breeding/testing material and machine parameters can be managed in Easy Breed, and field trial designs can be generated simply with a few mouse clicks. These field trials can be transferred to the MiniGIS software. MiniGIS allows for a GPS-based arrangement of the plots and transfers the GPS based field trials with the design information like fertilizer and seeding strength to the Dynamic Disc Plus precision spaced planter or to the electric drive Plot Motion equipped with Easy Plant and TopCon. After GIS reference seeding the actual sowing data can be transferred back to Easy Breed. This workflow ensures that the actual sowing corresponds to the sowing documented in Easy Breed and that further processes can be fed from this central data source.

Despite many digital techniques for data collection, manually collected scoring data on a variety of different traits remain very important. Increasingly, the paper field book is being replaced by handhelds with electronic field books or by scoring apps. No matter what the preference, in the end what matters is that the scores are correctly assigned to the plot and trait. To ensure this, the field books should be generated from the same central source as the sowing lists and, vice versa, the recorded scoring data should flow back to it automatically. This is exactly the case when using Wintersteiger Easy Breed and the scoring app smatrix (smatrix.systems; Dawin® GmbH, Siegburg). The data exchange takes place via the 'Data collection interface'. Field trials are managed within Easy Breed and field books are then transferred in DCI format to smatrix. Using smatrix, a voice-activated scoring can be performed, and the scoring data can be directly transferred back to Easy Breed.

A similar workflow exists between Easy Breed and the drones image analysis platform Alteia (alteia.com/industry/agriculture-forestry). The Easy Breed software can also transfer harvest lists to the Wintersteiger Easy Harvest combine harvester interface and, conversely, read in the recorded harvest data on a plot-by-plot basis.

Diehl S, Mayer M, Waack M, Basekow R (2023) Digital solutions in plant breeding. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 79-80. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

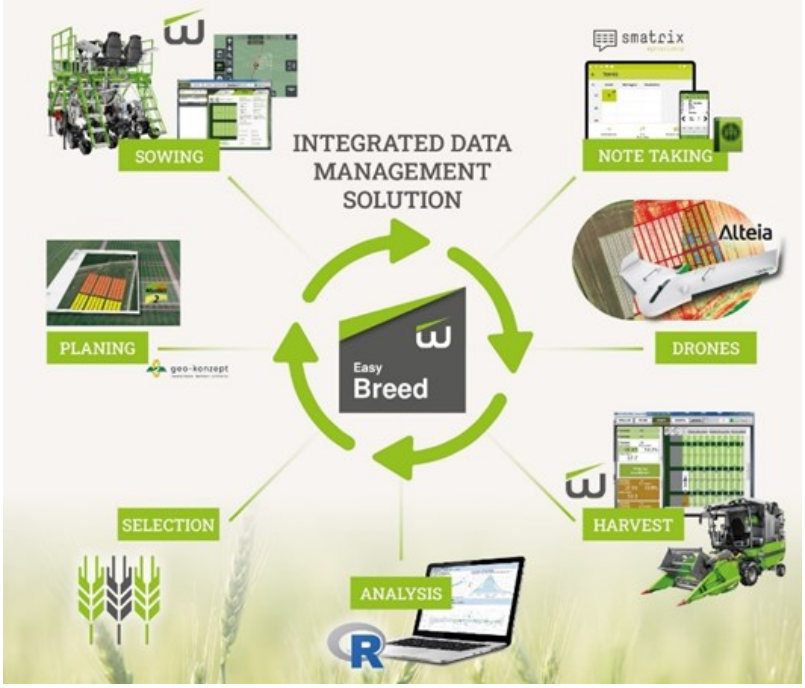

Figure 1 Easy Breed controls the process from field trial planning to data analysis and subsequent selection. The data collected in each step is integrated and can be passed on to subsequent steps.

Much is gained with such standardized workflows. It saves time and avoids errors. More importantly, the data is now available in a form that allows it to be merged, visualized, analyzed and used for decision making. A standard set of analysis functions is integrated in Easy Breed. This allows the data to be analyzed and used efficiently without time-consuming manual editing steps and even by 'non-coders'. For more extensive analyses, the Easy Breed software offers an interface to the statistical software R (r-project.org), which can be used to feed data from Easy Breed into your own workflows. This results in full flexibility without the time-consuming manual compilation and editing of data.

Our inventiveness, our acceptance of new things and the future will determine whether scoring in the future will take place in a virtual world or will be supported by augmented reality, whether artificial intelligence will significantly support us in selection or whether we will even be able to design our new varieties precisely using 'gene editing'. The prerequisite for all this is further digitization and, above all, standardization, and automation. This requires even closer coordination between breeders, technicians, hardware and software manufacturers, service providers, research institutions and other partners active in this industry.

Keywords

Automation · data management · interface · software · standardization

Further reading

Wintersteiger, Mobile data management & software [https://www.wintersteiger.com/en/Plant-Breeding-and-Research/Products/Product-range/Mobile-data-management]

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Enhancement of cocksfoot seed production

Lukas GAIER[1], Bernhard KRAUTZER[1], Wilhelm GRAISS[1], Andreas KLINGLER[1]

[1] HBLFA Raumberg-Gumpenstein, Institut für Pflanzenbau und Kulturlandschaft, Raumberg 38, 8952 Irdning-Donnerbachtal, Austria

(✉) lukas.gaier@raumberg-gumpenstein.at

Abstract

The production and propagation of site-adapted grass species are essential for providing high-quality forage in alpine areas. For Austrian agriculture, cocksfoot (*Dactylis glomerata*) represents one of the most important grass species in grassland. To increase this species' efficiency in seed production, we conducted an exact trial with the cocksfoot cv. 'Tandem' from 2016 to 2019. Under two different nitrogen fertilization management systems (single [N1] and split [N2] fertilization), we investigated the effects of a plant growth regulator, fungicides, and additional sulfur fertilization (individually and in a combination of all treatments) on seed yield and germination capacity. In terms of seed yield, a combination of all treatments resulted in a significant increase within the split fertilization regime compared to fertilization only. The use of fungicides and a combination of all treatments also significantly increased germination capacity in N1. Therefore, we recommend combining all crop protection treatments to optimize seed yield and germination capacity.

Keywords

Dactylis glomerata · germination capacity · nitrogen fertilization

Introduction

Around 67% of the agricultural land worldwide is covered by meadows and pastures. In the inner alpine areas and their peripheral regions, where arable farming and field forage cultivation are limited, permanent grassland is the predominant land use type (Buchgraber *et al.* 2011). The composition of grassland stands varies greatly depending on use and location, but it is generally a mixture of grasses, legumes, and forbs. In terms of yield and energy content of the forage, grasses are the most important. In addition to ryegrasses, cocksfoot (*Dactylis glomerata*) is particularly important in Austria. Although the forage quality of cocksfoot is lower than that of perennial ryegrass (*Lolium perenne*), it forms a higher yield in dry summer months and is also less susceptible to abiotic stress factors such as extreme weather conditions or moderately fertile soils. These traits are particularly strong in site-adapted varieties, but little is known about these varieties' seed production, especially in inner-alpine regions.

One of these varieties is the cocksfoot cv. 'Tandem', bred at HBLFA Raumberg-Gumpenstein and currently the only Austrian cocksfoot variety listed in the descriptive list of varieties. It is a leafy, medium-late variety with high digestibility and low susceptibility to foliar diseases (AGES, 2017).

Since no current studies are available on the potential increase in seed production of grasses, particularly cocksfoot, a field experiment was carried out from 2016 to 2019 in order to increase knowledge.

Material and methods

The trial was established in June 2016 in Gumpenstein, Austria (47°29 N, 14°06 E; 710 m a.s.l.) with cv. 'Tandem' and lasted three full growing seasons. Previously, the experimental site was used for a meadow foxtail (*Alopecurus pratensis*) seed propagation from 2013 to 2015. Planting was done on June 15, 2016, using broadcast seeding and a seed rate of 7 kg ha^{-1}. Harvesting was done with a Wintersteiger Classic plot combine (Wintersteiger, Ried im Innkreis, Austria) with a cutting width of 150 cm.

We investigated the effects of sulfur (S), the use of fungicide (F), and the use of a plant growth regulator (PGR), individually and in combination (S+F+PGR), under two different nitrogen fertilization management systems (*i.e*. N1: single application; N2: split application). Each nitrogen management system included a fertilization-only variant without further treatments, resulting in ten experimental variants. The concentration of active ingredients of the herbicides, growth regulator, and fertilization of the experimental plots can be found in Gaier *et al.* (2022).

In addition to quantitative seed yield, seed germination was also investigated. After threshing, seeds were dried in a drying chamber at 32°C for four days and cleaned with an Röber laboratory seed cleaner (Samatec Röber's Saatguttechnik & Maschinenbau, Bad Oeynhausen, Germany). The germination test was repeated six times and was carried out with a Copenhagen germination table with alternating day and night temperature levels of 15°C and 25°C. The whole process was designed according to the procedure specified by ISTA (2016). Statistical analysis was performed using SAS vers. 9.4 software (SAS Institute, Cary, NC), with the experiment arranged according to an α-design (Williams 2017).

Gaier L, Krautzer B, Graiss W, Klingler A (2023) Enhancement of cocksfoot seed production. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 81-82. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

Results and discussion

The combination of all crop protection treatments led to an increase in seed yield compared to the fertilization-only variant, regardless of fertilization management, although this was only significant in N2 (Table 1). Using PGR resulted in a significant increase in yield only in N2; this can be attributed to the low application rate, which also resulted in non-significant increases in the study of Gingrich & Mellbye (2001). In the individual trial years, the variants' differences were small and not significant in most cases. Differences between years were evident, with all variants achieving the highest yield in the second trial year.

The results of the germination capacity test (Table 2) showed a very high germination capacity for all trial variants, which was well above the legal minimum germination capacity of 80% (BAES 2017). No differences were observed between the trial variants in the first two trial years. In the third trial year, the fungicide-treated variants showed significantly higher germination rates compared to the other variants. Over the years, there were no differences in germination capacity in N2. In N1, the fungicide-treated variants had significantly higher germination capacity than the control variant. This can be explained by the protective effect of the fungicides, as they protect the plants from fungal diseases and thus lead to healthier and more vital seeds.

Acknowledgements

This study was financially supported by the Austrian Federal Ministry for Agriculture, Forestry, Regions and Water Management.

References

AGES (2017) Österreichische Beschreibende Sortenliste 2017 Landwirtschaftliche Pflanzenarten. Schriftenreihe 21/2017. Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien.

BAES (2017) Methoden für Saatgut und Sorten gemäß § 5 Saatgutgesetz 1997 BGBl. I Nr. 72/1997 idgF. - Anforderungen an die Beschaffenheit und Methoden zur Bestimmung der Beschaffenheit von Saatgut. Sorten- und Saatgutblatt, 25. Jhg., Schriftenreihe 17, Sondernummer 54. Bundesamt für Ernährungssicherheit (BAES), Wien.

Buchgraber K, Schaumberger A, Pötsch E (2011) Grassland farming in Austria - Status quo and future prospective. In: Pötsch EM, Krautzer B, Hopkins A (eds), Proc 16th Symp Eur Grassland Fed, Grassland Farming and Land Management Systems in Mountainous Regions, 29-31 Aug, Gumpenstein, Austria. Grassland Science in Europe 16:13-24.

Gaier L, Krautzer B, Graiss W, Klingler A, Pötsch EM (2022) Effect of fertilization and plant protection treatments on seed yield and seed quality of orchardgrass (*Dactylis glomerata* L.). Grassland Sci 68:277-285. DOI: 10.1111/grs.12362

Gingrich G, Mellbye ME (2001) The effect of plant growth regulators on seed yields of grass crops. In: Young III WC (ed), 2000 Seed Production Research at Oregon State University USDA-ARS Cooperating, pp 37-39. Oregon State University Extension Service. [https://ir.library.oregonstate.edu/concern/technical_reports/mp48sd352; accessed 3 Mar 2023]

ISTA (2016) International Rules for Seed Testing, Vol. 2016. International Seed Testing Association (ISTA), Wallisellen, Switzerland.

Williams ER (2017) Alpha-Designs. Wiley StatsRef: Statistics Reference Online. DOI: 10.1002/9781118445112.stat07333.pub2

73. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2022, Raumberg-Gumpenstein, Österreich

Conserving Crop Wild Relatives as new breeding resources

Sylvia VOGL[1], Paul FREUDENTHALER[1]

[1] Austrian Agency for Health and Food Safety (AGES), Wieningerstr. 8, 4020 Linz, Austria

(✉) sylvia.vogl@ages.at

Abstract

Crop Wild Relatives (CWR) are said to be the wild 'cousins' of our cultivated crops (cwrdiversity). There is a wide range of wild plant species related to our crops that provide a great diversity of traits that could be used to adapt and improve our crops. As cultivated genepools get more and more depleted, breeders will need to use alternative breeding resources. Especially in terms of combating with the adverse impacts of climate change on our agricultural production systems CWR will become more and more important.

CWR are genetically related to our cultivated crops and can belong to the

- same species
  - 'subsp.' (subspecies), *e.g*. *Daucus carota* subsp. *sativus* (cultivated), subsp. *carota* (wild carrot)
  - 'var.' (variety)
  - 'f.' (form)
- same genus
  - different species but related, *e.g*. *Avena sativa* (oat) and *A. sterilis* (animated oat)
- same family
  - different but related genus, *e.g*. *Triticum aestivum* (wheat) and *Aegilops tauschii* (Tausch's goat grass)

They can, more or less easily, be crossed with domesticated crops (same genepool) and have been used to improve crops since the beginnings of agriculture. There has already been done a lot of effort to classify CWR related to their closeness of relationship. For determining the ease by which CWR species can be used in breeding efforts, two major systems exist. The Harlan & de Wet (1971) genepool concept divides CWR species into primary, secondary and tertiary genepools based on how easy they can be used for breeding. However, this concept has not been applied to all crops. Therefore the taxon group concept published by Maxted *et al*. (2006) can be used as a proxy for relative crossability.

Acording to the Harlan & de Wet (1971) concept, the primary genepool (GP1) includes the closest related CWR. They can be directly mated with the related crop to produce strong and fertile progeny. They often belong to the same species, so it's mostly subspecies or varieties, but also related species. For example the GP1 of *Helianthus annuus* (sunflower) includes not only wild varieties of *H. annuus* but also the *H. winterii* (Winter's sunflower).

CWR from the secondary genepool (GP2) are more distinct from the cultivated species but still so closely related that they can cross to at least some extent to produce some fertile offspring. However, hybrids are partly sterile or just weaklings and those CWR are more difficult to use because of reproductive barriers. For example, the wild relatives in GP2 of bread wheat *Triticum aestivum* (hexaploid), *Aegilops tauschii* and *Ae. speltoides* are diploid. Those mismatches create difficulties for breeders.

CWR from the tertiary genepool (GP3) are even more distantly related and must be coaxed with the use of specific breeding techniques, such as embryo rescue or "bridge crosses" with members of the secondary genepool. The resulting progeny is often sterile. Anything farther away and you'll need biotechnology to transfer genes. Examples for wild relatives in GP3 of *T. aestivum* are *Thinopyrum elongatum*, *Elymus sibiricus*, *Leymus mollis* and *Agropyron cristatum*.

The reasons for breeders to use CWR are diverse. Genetic diversity in CWR is much higher than in cultivated varieties (Tanksley & McCouch, 1997). CWR evolve in the wild, untended by humans. Therefore, they are exposed to the changing nature, *e.g*. climatic changes (*e.g*. drought, heavy rainfall, etc.) or (new) diseases or pests. In order to survive they have to develope traits adapting to those natural conditions, *e.g*. drought tolerance or pest resistance. Those traits are interesting for our cultivated crops as well and therefore need to be sustainably conserved.

On the one hand, information about and conservation of CWR populations is rare (more than 90% are insufficiently safeguarded in the world's gene banks). On the other hand, *in-situ* CWR populations are valuable resources for science and breeding. That's why they need to be conserved properly and need to be made available.

Genebanks provide experience and resources for a standardized conservation. Genebanks are national institutions that follow international regulations (*e.g*. FAO genebank standards). Many states have made a legal commitment to preserve their genetic resources to ensure that their genetic material is safely conserved and available for people to use. Plant genetic resources „means any genetic material of plant origin of actual or potential value for

Vogl S, Freudenthaler P (2023) Conserving Crop Wild Relatives as new breeding resources. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 73rd Conference 2022, 21-23 November, Raumberg-Gumpenstein, pp 83-84. University of Natural Resources and Life Sciences, Vienna, Austria. DOI: 10.5281/zenodo.7875597

food & agriculture.“ (FAO, 2009). In this context CWR have to be included in the conservation, as they have an actual and potential value for our food and agricultural system.

In order to conserve CWR properly several steps need to be untertaken:

- conservation *in-situ* in order to keep the genepool variable and adaptable to the changing nature
- as well as *ex-situ* conservation in genebanks in order to make the material available
- identification of CWR and their related crop
- checklist of priority crops
- favorable traits in these species
- crossability of the CWR (genepool)
- threat status (protection status, existing conservation actions, …)
- focus on populations that can be made available to users in principle
- information about how to obtain material (terms, conditions,…)
- implementation of a database
- prebreeding

Keywords

Diversity conservation · CWR · genepool · plant genetic resources

Internet resources

Bioversity International - https://www.bioversityinternational.org/cwr/

ECPGR CWR Working Group - https://www.ecpgr.cgiar.org/working-groups/crop-wild-relatives

Farmer‘s Pride - https://more.bham.ac.uk/farmerspride/key-documents/crop-wild-relatives/

Global Diversity Trust - https://www.cwrdiversity.org

References and further reading

Castañeda-Álvarez NP, Khoury CK, Achicanoy HA, Bernau V, Dempewolf H, *et al*. (2016) Global conservation priorities for crop wild relatives. Nat Plants 2:16022. DOI: 10.1038/nplants.2016.22

Dempewolf H, Baute G, Anderson J, Kilian B, Smith C, *et al*. (2017) Past and future use of wild relatives in crop breeding. Crop Sci 57:1070-1082. DOI: 10.2135/cropsci2016.10.0885

FAO (2009) International Treaty on Plant Genetic Resources for Food and Agriculture. Food and Agriculture Organization of the United Nations (FAO), Rome. [http://www.fao.org/3/i0510e/I0510E.pdf; accessed 3 Mar 2023]

Harlan JR, de Wet JMJ (1971) Toward a rational classification of cultivated plants. Taxon 20:509-517. DOI: 10.2307/1218252

Labokas J, Maxted N, Kell SP, Magos Brehm J, Iriondo JM (2018) Development of national crop wild relative conservation strategies in European countries. Genet Resour Crop Evol 65:1385-1403. DOI: 10.1007/s10722-018-0621-x.

Magos Brehm J, Kell SP, Thormann I, Gaisberger H, Dulloo ME, *et al*. (2019) New tools for crop wild relative conservation planning. Plant Genet Resour 17:208-212. DOI: 10.1017/S1479262118000527

Maxted N, Ford-Lloyd BV, Jury S, Kell S, Scholten M (2006) Towards a definition of a crop wild relative. Biodiv Conserv 15:2673-2685. DOI: 10.1007/s10531-005-5409-6

Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: Unlocking genetic potential from the wild. Science 277:1063-1066. DOI: 10.1126/science.277.5329.1063

www.saatgut-austria.at