IBGS 13

13th INTERNATIONAL BARLEY GENETICS SYMPOSIUM

JULY 3RD - 7TH, 2022 RIGA, LATVIA

ABSTRACT E-BOOK

Organized by Institute of Agricultural Resources and Economics (AREI)

AREI

13TH INTERNATIONAL BARLEY GENETICS SYMPOSIUM (IBGS 13)

13th International Barley Genetics Symposium (IBGS 13) takes place from **July 3 -7, 2022**, in **Riga**, in the capital of **Latvia** that is not only the crown jewel of Latvia, but also of the Baltics.

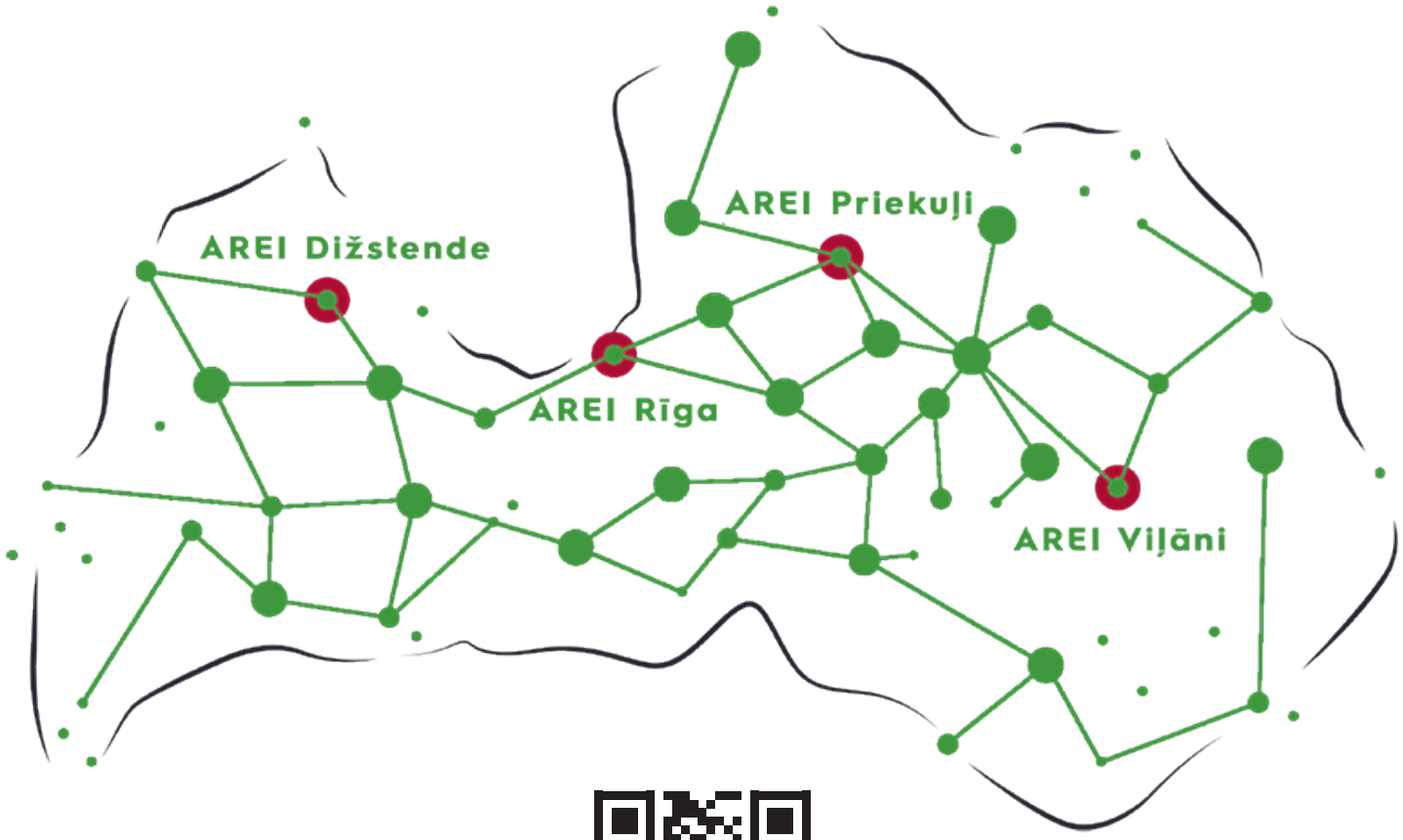
The International Barley Genetics Symposium, usually held every 4 years, is the **single most important scientific event for barley researchers and breeders all over the world**. These meetings have never been held in the Baltic countries before and the last meeting in Europe was in 2004. Due to covid-19 pandemics, IBGS was postponed for 2 years from 2020 to 2022.

For the international barley community this is a high profile meeting and local organisers are fortunate to be hosting it in Latvia. The venue for the symposium is the **Academic Centre of University of Latvia** with a field day held at **Priekuļi Research Centre of the Institute of Agricultural Resources and Economics (AREI)** around 100 km from the capital Riga.

IBGS 13 program comprises **10 various topics.**

INSTITUTE OF AGRICULTURAL RESOURCES AND ECONOMICS (AREI)

Institute of Agricultural Resources and Economics (AREI) is the leading Latvian institute for research on crop breeding and rural economics, with a history of more than 100 years.
AREI scientists are focusing on crop breeding, including spring barley breeding in two locations (Stende and Priekuļi), research on various crop management technologies, studies on crop quality, as well as the development of sustainable rural areas. Besides, AREI provides economic analysis for the agricultural, food production and fisheries sectors.
AREI activities have been performed in four locations in Latvia making it possible to organize the research in various environments.



INSTITUTE OF AGRICULTURAL RESOURCES AND ECONOMICS

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A = agriculture
R = research
E = effectivness
I = innovations

IBGS LOCAL ORGANIZING COMMITTEE (LOC)

NAME	INSTITUTION, COUNTRY	EXPERTISE
Nils Rostoks, chair	University of Latvia, Latvia	Molecular genetics, association mapping, disease resistance
Alan H. Schulman, vice chair	University of Helsinki, Finland	Genomics, abiotic stress response
Māra Bleidere	Institute of Agricultural Resources and Economics, Latvia	Barley breeding
Linda Legzdiņa	Institute of Agricultural Resources and Economics, Latvia	Barley breeding
Isaak Rashal	University of Latvia, Latvian Society for Geneticists and Breeders, Latvia	Genetics and breeding
Søren Kjærsgaard Rasmussen	University of Copenhagen, Denmark	Barley grain quality and end use; molecular breeding
Mats Hansson	Lund University, Sweden	Barley genetics, mutants
Duane E. Falk	University of Guelph, Canada	Barley breeding methodology
Ülle Tamm	Estonian Crop Research Institute, Estonia	Barley breeding
Kristiina Laanemets	Estonian Crop Research Institute, Estonia	Barley breeding
Algė Leistrumaitė	The Lithuanian Institute of Agriculture, Lithuania	Barley breeding
Ahmed Jahoor	Nordic Seed and Swedish Agricultural University	Genome based barley breeding

IBGS INTERNATIONAL ORGANIZING COMMITTEE (IOC)

NAME	INSTITUTION, COUNTRY	EXPERTISE
Guoping Zhang	Zhejiang University, China	Barley breeding and crop physiology
Nils Steins	Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany	Plant genetics, breeding of crop plants
Euclydes Minell	Brazilian Agricultural Research Corporation (EMBRAPA), Brazil	Barley breeding
Bryan Harvey	University of Saskatchewan, Canada	Barley breeding
Olga Kovaleva	N.I. Vavilov All-Russian Research Institute of Plant Industry, Russia	Research on genetic diversity
Wayne Powell	Scotland's Rural College, United Kingdom	Physiology and Genetics
Kazuhiro Sato	Okayama University, Japan	Barley genetics
Alan Schulman	University of Helsinki, Finland	Genomics, abiotic stress response
Flavio Capettini	Field Crop De-velopment Centre, Canada	Barley breeding
Chengdao Li	Western Barley Genetics Alliance, Australia	Genetics and breeding
Kevin Smith	University of Minnesota, USA	Barley breeding and genetics

IBGS HISTORY IN SHORT

IBGS SERIES	YEAR	LOCATION
1st	1963	Wageningen, The Netherlands
2nd	1969	Pullman, United States
3rd	1975	Munich, Germany
4th	1981	Edinburgh, Scotland
5th	1986	Okayama, Japan
6th	1991	Svalöv, Sweden
7th	1996	Saskatoon, Canada
8th	2000	Adelaide, Australia
9th	2004	Brno, Czech Republic
10th	2008	Alexandria, Egypt
11th	2012	Hangzhou, China
12th	2016	Minneapolis, United States
13th	2022	Riga, Latvia

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July 3, Sunday

14.00 - 17.00 Registration and poster set up: University of Latvia, Academic Centre, the House of Nature (Riga, Jelgavas street 1)
17.30-20.30 Welcome reception: Radisson Blu Daugava Hotel Riga, (Rīga, Kugu street 24)

July 4, Monday

7:30 - 8:30 Registration and Poster setup: University of Latvia, Academic Centre, the House of Nature (Riga, Jelgavas street 1)

OPENING SESSION
8:30 - 9:00 Representatives of IBGS13 LOC, IBGS IOC, the Institute of Agricultural Resources and Economics, the Latvia Ministry of Agriculture, University of Latvia, Ministry of Education and Science

The barley genome: from reference genome to structure and function (Chair **Nils Stein**, Gatersleben, DE)
9:00 - 9:30 Keynote: Martin Mascher, IPK Gatersleben, Germany - ***Pangenomics in barley and beyond***
9:30 - 9:50 Einar Baldvin Haraldsson - A new genomic resource for crop improvement: the complete reference genome of Hordeum erectifolium using long-read genomic and transcriptomic sequencing
9:50 - 10:10 Alessandro Tondelli - A gene duplication at the Blp1 locus is associated with the black grain phenotype in barley
10:10 - 10:30 Martin Kovacik - Developing an atlas of gene expression during barley grain development

10:30 - 11:00 Coffee break

Biotic stresses under Climate Change (Chair **Robert S. Brueggeman**, Washington State University, USA)
11:00 - 11:30 Keynote: Brian J. Steffenson, Department of Plant Pathology, University of Minnesota, USA - ***Pan-genome enabled disease resistance gene discovery in wild barley***
11:30 - 11:50 Karl Effertz - Rpt5 encodes a receptor-like protein that provides the broadest and most effective net form net blotch (Pyrenophora teres f. teres) resistance in barley
11:50 - 12:10 Molly Bergum - Functional diversification of a barley receptor kinase involved in immunity to wheat stripe rust
12:10 - 12:30 Ping Yang - Convergent mechanisms of host susceptibility factors assisting genetic improvement of the bymovirus resistance in barley and wheat

12:30 - 13:30 Lunch break

Abiotic stresses under Climate Change (Chair **Ernesto Igartua,** EEAD-CSIC, ES)
13.30 - 14:00 Keynote: Sergey Shabala, School of Agricultural Science, University of Tasmania, AU – ***Cell-based phenotyping approach for improving abiotic stress tolerance in barley***
14:00 - 14:20 "Eyal Fridman - Needles in the stressed hay: Can wild barley alleles contribute to yield
abiotic tolerance?"
14:20 - 14:40 Agata Dsazkowska-Golec - The transcriptome landscape of barley under drought stress reveals insights into the role of alternative splicing in adaptation to stress
14:40 - 15:00 Maitry Paul - Drought response and recovery in barley: a role for autophagy and its link to retrotransposon expression dynamics.

15:00 - 15.30 Coffee break

Barley breeding and New Breeding Techniques (NBTs): a way forward? (Chairs **Wendy Harwood**, John Innes Centre, UK; **Kevin Smith,** University of Minnesota, USA)
15.30 - 16:00 Keynote: Dr. Jochen Kumlehn, IPK Gatersleben, DE - ***Site-directed genome modification in barley - methods and applications***
16:00 - 16:20 Julian Garcia-Maldonado - Genomic-assisted sparse multi-location testing to increase genetic gain in barley
16:20 - 16:40 Tom Lawrenson and Wendy Harwood - New tools for barley genome editing
16:40 - 17:00 Alan Schulman - Current status and future prospects for NBT regulation in Europe

17:00 - 18:00 Poster session I Coffee&Snaks

18.00 - 19.30 EVENING WORKSHOP ***1. Funding of a barley research: further strategic directions (moderator Alan Schulman) Summary on the funding picture going forward beyond the Horizon 2020 and some strategic directions defined by European Plant Science Organisation (EPSO); Barley Community Initiative (moderator Robbie Waugh):***

introduce and discuss the Barley Community Initiative (BCI: https://wheat.pw.usda.gov/GG3/content/initiative-re-galvanise-international-barley-research-community) and discuss how our community could actively develop from where we are now. A number of us could certainly give overviews of whats been done so far, stimulate discussion and seek commitment from a 'broader church' than currently subscribe.

Dinner (on your own)

July 5, Tuesday

Barley breeding: success stories (Chair **Ahmed Jahoor,** Nordic Seed, DK)

8:30 - 9:00 **Keynote: Birger Eriksen,** Sejet Plant Breeding, DK – ***Barley cultivar development in Europe – success in the past and possible changes in the future"***

9:00 - 9:20 **Alexander Strube** - New developments in IP in plant breeding – a golden or a dark age for innovation?

9:20 - 9:40 **Emmanuelle Dyrszka** - Breeding for the producibility of female parental lines in hybrid barley

9:40 - 10:00 **Pernille Merete Sarup** - Putting millions of markers and thousands of yield plots to the test: Genomic prediction of 2-row spring barley

10:00 – 10:30 Coffee break

Genetics and breeding of biomass (Resources use efficiency/RUE) (Chair **Dr. Klaus Pillen**, Martin Luther University, Halle-Wittenberg, Halle, DE)

10:30 - 11:00 **Keynote: Daniel Julio Miralles,** University of Buenos Aires, AR – ***What were the physiological attributes modified by the breeders***

11:00 - 11:20 **Lana Shabala** - Physiological and agronomical aspects of potassium use efficiency (KUE) in barley

11:20 - 11:40 **Paolo Pesaresi** - The barley mutant happy under the sun 1 (hus1): An additional contribution to pale green crops

11:40 - 12:00 **Sonia Negrao** - Diving into the genetic diversity of the European Herltage Barley collecTion (ExHIBiT)

12:00 - 13:00 Lunch break

Barley end uses: from food and feed to malting, brewing, and novel products (Chairs **Kim Hebelstrup**, Aarhus University, DK; **Christoph Dockter**, Carlsberg Research Laboratory, DK)

13:00 - 13:30 **Keynote: Henrik Brinch Pedersen**, Aarhus University, DK – ***New potentials of the barley grain***

13.30 - 13.50 **Markus Herz** - Assessment of winter barley for feeding quality traits to breed for improved protein utilization in pig nutrition

13.50 - 14.20 **Keynote: Christoph Dockter**, Carlsberg Research Laboratory, DK – ***Acceleration with a purpose – ultrafast barley trait*** **development** ***for sustainable beer production***

14:20 - 14.40 **Leona Leišová-Svobodová** - Molecular markers for malting barley breeding for PGI Czech beer

14.40 - 15.10 Coffee break

SPONSOR PRESENTATION

15.10 - 15.40 **Miguel Sanchez-Garcia** - The Global Barley Breeding Program in the new OneCGIAR: New strategies to breed barley in and for the Developing World

Flash & Dash presentations (Chair **Outi Manninen**, Boreal Plant Breeding Ltd., FI)

15:40 - 17:00 Short oral presentation from students and postdocs

17:00 - 18:00 Poster session II
Coffee&Snaks

18.00 - 19.30 **EVENING WORKSHOP 2. Barley genetic resources.** ***Welcome (Chair. Dr. Søren K. Rasmussen, University of Copenhagen, DK) Jan T. Svensson, Nordic Genetic Resource Center, Sweden: Barley collection at NordGen Andrea Visioni, Rabat, Morocco: Barley collection at ICARDA Nils Stein, Gatersleben, Germany: Barley Collection at IPK Ernesto Igartua Arregui, CSIC, Zaragoza, Spain: Mediterranean landraces, GENDIBAR Fluturë Novakazi, SLU, Sweden: Origin of Icelandic barley germplasm Chengdao Li, Murdoch University, Australia: Barley germplasm in Australia***

July 6, Wednesday

Genetic and database resources: harnessing diversity (Chairs **Dr. Robbie Waugh**, James Hutton Institute, Dundee, Scotland, UK; **Dr. Mats Hansson**, Lund University, SW)"

8:30 - 9:00 **Keynote: Nils Stein,** IPK Gatersleben, DE – ***At the dark side of barley diversity – the gap between discovery and user-friendly access***

9:00 - 9:20 **Asis Shrestha** - The double round

robin population unravels the genetic architecture of grain size in barley
9:20 - 9:40 Outmane Bouhlal - CGIAR BARLEY BREEDING TOOLBOX: A diversity panel to facilitate breeding and genomic research in the Developing World
9:40 - 10:00 Ronja Wonneberger - Comprehensive expression atlas of six tissues coupled with high density SNP data provides a resource for studies and characterization of European two-rowed spring barleys and identified a major haplotype switch on chromosome 5H

10.00 – 10.30 Coffee break

Genetic and database resources: harnessing diversity (continued)
10.30 - 10.50 Agatha Walla - Quest for an elusive developmental gene in barley
10.50 - 11.10 Congcong Jiang - A reference-guided TILLING by amplicon-seq platform supports forward and reverse genetics in barley

Morphology, phenology, and development (Chair **Dr. Laura Rossini**, University of Milan, Milan, IT)

11.10 - 11.40 Keynote: Maria von Korff Schmising, Institute of Plant Genetics, Heinrich-Heine-University, Düsseldorf, DE – ***Inflorescence development and floral abortion under stress in barley***
11:40 - 12.00 Ravi Koppolu - Spikelet determinacy as a factor for improving grain yield potential in Triticeae crops

12:00 - 13.00 Lunch break

Morphology, phenology, and development (continued)

13.00 - 13.20 Luke Ramsay - An induced mutation in HvRecql4 increases overall recombination and restores fertility in a barley HvMlh3 mutant background
13.20 - 13.40 Ivan Acosta - The role of auxin in the starch production of barley pollen
13.40 - 14.00 Vanda B. Marosi - Systematic Comparison of Barley and Wheat Transcriptomes Reveals Evolutionary Insights of Flower Development
14.00 - 14.20 Silvio Salvi - Dissecting barley root development and tropism by cloning chemical-induced mutants

14:20 - 15:00 Coffee break

Baltic and Nordic barley: a regional perspective (Chair **Søren K. Rasmussen,** University of Copenhagen, DK)

15.00 - 15.30 Keynote: Therése Bengtsson, Swedish University of Agricultural Sciences, Alnarp, SE – ***Together we are stronger- United forces to promote Nordic pre-breeding in spring barley***
15.30 - 15.50 Alge Leistrumaite - Barley in Lithuania: a century long breeding story
15.50 - 16.10 Mara Bleidere - Centenary retrospect and outlook of spring barley breeding and research in Latvia
16.10 - 16.30 Magnus Göransson - What is unique about Icelandic barley?

16.30 - 18.00 Poster session III Coffee&Snaks

18.00 - 19.30 EVENING WORKSHOP 3.
Honoring barley research coryphaei (moderator Mats Hansson); IBGS Bussiness session: IBGS13 statistics&venue of the next symposium (moderators representative from LOC and IOC)

Dinner (on your own)

July 7, Thursday

8.00 - 8.15 Boarding busses. University of Latvia, Academic Centre, the House of Nature (Riga, Jelgavas street 1)

8.15 - 10.00 Travel to the field day: Rīga - Priekuļi
IBGS13 Barley Field Day
10.00-10.30 Arrival to the field, refreshments
10.30- 12.30 Tour through the nurseries in groups
12.30-13.00 Free time to go back to the most interesting material

13.00 - 14.00 Lunch break

14.00 - 18.00 Excursion: guided town tour in Cēsis

18.00 - 22.00 Farewell party: the Castle of Cēsis

22.00 - 24.00 Returning to Riga
AREI organizers:
Juris Multiņš +37126511022
Andra Ūdre +37129441332
Agnese Hauka +371280374741 ***Emergency 112***

THE GLOBAL BARLEY BREEDING PROGRAM IN THE NEW ONE CGIAR: NEW STRATEGIES TO BREED BARLEY IN AND FOR THE DEVELOPING WORLD

Miguel Sanchez-Garcia
Biodiversity and Crop Improvement Program (BICP), International Centre for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco
m.sanchez-garcia@cgiar.org

Co-Authors:

Andrea Visioni
Biodiversity and Crop Improvement Program (BICP), International Centre for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco

Seid K. Ahmed
Biodiversity and Crop Improvement Program (BICP), International Centre for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco

Zakaria Kehel
Biodiversity and Crop Improvement Program (BICP), International Centre for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco

Michael Baum
Biodiversity and Crop Improvement Program (BICP), International Centre for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco

ICARDA has the global mandate within the CGIAR system to breed barley for the developing World. As such, more than 250 barley varieties of CGIAR origin have been released in 46 countries and many more are being used as parents in breeding programs Worldwide.
The Global Barley Breeding program is continuously trying to improve the genotypes provided to its collaborators. Thus, following the recommendations of the Excellence in Breeding (EiB) Platform, the program has adopted a strategy based on the systematic use of product profiles, rapid generation advancement and the extensive use of new genomic and phenotyping tools. The aim is to rapidly and efficiently develop new genotypes that maintain the successful traits of key cultivars and incorporate new and better traits to cope with Climate Change. Resistance to new insect pests and diseases, tolerance to more frequent and intense drought and heat waves, increasing soil degradation or salinity and better end-use quality are some of the main challenges. For it, in collaboration with the National Agricultural Research Programs (NARS), new product profiles are designed and grouped in 4 MegaProduct Lines: Feed Barley for Arid and Semi-Arid regions, Food and Fodder Barley, Feed and Forage Barley for Favorable Environments and Malt and Fodder Barley.

Following the product profile scheme, breeding strategies have been designed for each of the MegaProduct Lines. The traditional and effective use and deployment of the barley genetic diversity hosted at ICARDA genebank is strengthened using genomic assisted Focused Identification of Germplasm Strategy (FIGS). Also, to reduce the time needed from crossing to testing new genotypes in yield trials the program makes use of the new ICARDA Speed Breeding Platform (Rabat, Morocco). In this facility, in addition to reaching 4 generations per year, new protocols allow to couple fast cycling with disease and quality testing in new Disease assisted- and NIR assisted-Speed Breeding strategies.

To increase selection intensity and accuracy the program combines new genomic assisted testing strategies with the use of key-location testing sites in Morocco, Egypt, Lebanon and India. Each of these sites represent a target population of environments and are hot-spots for major biotic and abiotic constraints.
Genomic approaches are used both for parental selection, in multi-trait predictions in simulated progenies, and for performance predictions in generation advancement. Low density genotyping is used in combination with sparse multi-location testing to increase the number of lines tested at preliminary yield trial level while predicting multiple traits of planted and non-planted lines across locations. In these, the prediction abilities for yield of the genomic GxE models range from r=0.20 to 0.55.
These new strategies converge in strong collaborative breeding and research endeavors between the program and the NARS to bring the new genotypes rapidly to farmers' fields.

KEYWORDS: Product Profile, Speed Breeding, Genomic Predictions, Developing countries, Genotype by Environment, drought, heat, diseases, insect pests, breeding

THE BARLEY GENOME: FROM REFERENCE GENOME TO STRUCTURE AND FUNCTION

PAN-GENOMICS FOR BARLEY IMPROVEMENT

Martin Mascher
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Seeland, Germany
mascher@ipk-gatersleben.de

Pan-genomic studies aim at representing the entire sequence diversity within a species to provide useful resources for evolutionary studies, functional genomics and breeding of cultivated plants. Cost reductions in high-throughput sequencing and advances in sequence assembly algorithms have made it possible to create multiple annotated reference genome sequences along with a catalogue of all forms of genetic variations in plant species with large and complex genomes. In this talk, we will summarize our work in barley pan-genomics: the selection of representative core sets by genebank genomics, the assembly of chromosome-scale reference sequences, and the discovery of structural variants associated with agronomic traits. Future challenges will be the development of pan-genome interfaces for easy access by breeders and geneticists and the construction of a genus-wide pan-genome to make the wild relatives of barley more amenable to (pre-)breeding.

KEYWORDS: pan-genome, structural variation, genebank genomics, association genetics, genome sequence assembly

A NEW GENOMIC RESOURCE FOR CROP IMPROVEMENT: THE COMPLETE REFERENCE GENOME OF HORDEUM ERECTIFOLIUM USING LONG-READ GENOMIC AND TRANSCRIPTOMIC SEQUENCING

Einar Baldvin Haraldsson
Heinrich Heine University Düsseldorf, Germany
einar.haraldsson@hhu.de

Co-Authors:

Maria von Korff Schmising, Jan Piotr Buchmann
Heinrich Heine University Düsseldorf, Germany

Martin Mascher
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Seeland, Germany

Helena Toegelová, Zuzana Tulpová, Hana Šimková
Institute of Experimental Botany of the Czech Academy of Sciences, Olomouc, Czech Republic

To be or not to be, annual-perennial.

Crop genetic diversity is a cornerstone of a productive, robust, and adaptive agriculture. Now that we are facing increasing environmental disruptions through climate change, genetic diversity has become more crucial for resilient and agriculturally sustainable crops. However, only a few annual crops represent over 85% of our caloric intake. A major drive in rethinking the current agricultural system is the creation of a low-input, robust, and adaptive perennial crops. With modern technological advances in genetic transformation we can now employ a more direct approach by genome editing or direct gene transfer between species, but first new genomic resources to discover unique and novel loci are needed.

We have now generated a complete reference genome of Hordeum erectifolium, a perennial grass species in the Hordeum genus and a wild relative of the common annual crop barley (H. vulgare). Hordeum erectifolium has a 4.4 Gbp genome and is an endemic species to a single location in Argentina. We constructed the genomic base with long-read Oxford Nanopore Technologies and for structural gene annotation of the transcriptome we employed Pacbio Isoseq.

This genome is the first complete reference of a perennial species within the Hordeum genus and the second reference genome after barley. It will serve as a platform to discover new genetic adaptations and give us a first glance into the genomics of a perennial Hordeum species.

KEYWORDS: genomes, bioinformatics, transcriptomics, genomic resource

A GENE DUPLICATION AT THE BLP1 LOCUS IS ASSOCIATED WITH THE BLACK GRAIN PHENOTYPE IN BARLEY

Alessandro Tondelli
CREA, Research centre for Genomics and Bioinformatics, Fiorenzuola d'Arda, Italy
alessandro.tondelli@crea.gov.it

Co-Authors:

Davide Guerra, Gabriele Viottini, Luigi Cattivelli
CREA, Research centre for Genomics and Bioinformatics, Fiorenzuola d'Arda, Italy

Chengdao Li
Western Barley Genetic Alliance, Murdoch University, Perth, WA, Australia

Ali Naz
Department of Plant Breeding, University of Bonn, Bonn, Germany

Martin Mascher, Nils Stein
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Seeland, Germany

Due to its wide ecological distribution, barley is considered a useful source of genetic diversity for adaptive traits. For instance, the black colour of barley grains, caused by the accumulation of phytomelanin in the pericarp and husk, has been suggested to result from environmental adaptation to biotic and abiotic stresses. By exploiting whole exome sequencing data from a collection of barley landraces of different origins (WHEALBI panel), the Black lemma and pericarp (Blp) locus responsible of the black grain phenotype was mapped at gene-resolution level on chromosome 1H and a gene coding for a Purple Acid Phosphatase (PAP) was proposed as the best candidate for the trait. Mining exome reads for heterozygous calls and depth of coverage at the locus revealed a duplication of the PAP gene in black barley genotypes, suggesting a possible neofunctionalization, in agreement with the dominant inheritance of the locus. The PAP gene duplication was confirmed by exploiting the barley pan-genome and by developing paralog-specific genomic and expression markers. An increase in the expression level of the duplicated PAP paralog was observed in black barleys during spike maturation. Screening of mutagenized populations of black barleys is underway. A correlation between the origin of black barleys and the higher incidence of solar radiation has been observed, suggesting that the accumulation of phytomelanin in the lemma and pericarp might provide an adaptive advantage in such environments. Other than representing a significant improvement on the cloning of Blp, our study is a valuable example on the integration of genetic and genomic resources for the identification of structural variants responsible of heritable phenotypes.

KEYWORDS: Bl'

DEVELOPING AN ATLAS OF GENE EXPRESSION DURING BARLEY GRAIN DEVELOPMENT

Martin Kovacik
Institute of Experimental Botany, Czech Acad Sci, Centre of the Region Haná for Biotechnological and Agricultural Research, CZ-779 00 Olomouc, Czech Republic

kovacik@ueb.cas.cz

Co-Authors:

Anna Nowicka, Ales Pecinka, Jana Zwyrtkova
Institute of Experimental Botany, Czech Acad Sci, Centre of the Region Haná for Biotechnological and Agricultural Research, CZ-779 00 Olomouc, Czech Republic

Isaia Vardanega, Rüdiger Simon
Institute for Developmental Genetics, Heinrich-Heine-University Düsseldorf, Düsseldorf 40225, Germany

Nicholas J. Provart
Department of Cell and Systems Biology/Centre for the Analysis of Genome Evolution and Function, University of Toronto, 25 Willcocks St., Toronto, ON M5S 3B2, Canada

Cereal seeds are an important source of food, feed and raw materials for biofuels and other technical products. We use barley (Hordeum vulgare) to study molecular and cellular mechanisms governing seed development. To provide comprehensive spatiotemporal information about barley grain developmental processes, we performed an RNA-seq-based transcriptomic study of different seed tissues (embryo, endosperm, seed maternal tissues) from 4 until 32 days after pollination. Analysis of differential gene expression and gene clustering based on their expression profiles revealed the major biological processes ongoing in different grain tissues at different stages. Gene co-expression network and motif enrichment analysis pointed out specific groups of genes and transcription factors with possible impacts on the regulation of endosperm development. We also defined a set of tissue-specific marker genes and families of transcription factors that can help understand the major pathways controlling barley seed development. Altogether, our atlas of gene expression during barley grain development will be a useful resource for both basic research scientists and also cereal breeders.

KEYWORDS: seed, embryo, endosperm, RNA-seq, development

CHROMOSOME-SCALE AND HAPLOTYPE-RESOLVED SEQUENCE ASSEMBLY OF HORDEUM BULBOSUM GENOMES

Jiawu Feng
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Seeland, Germany
fengj@ipk-gatersleben.de

Co-Authors:

Maria Cuacos, Hélène Pidon, Yi-Tzu Kuo, Jörg Fuchs, Axel Himmelbach, Jochen Kumlehn, Stefan Heckmann, Andreas Houben, Frank Blattner
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Seeland, Germany

Thomas Lux, Heidrun Gundlach, Manuel Spannagl
Plant Genome and Systems Biology (PGSB), Helmholtz Center Munich, German Research

Nils Stein, Martin Mascher
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Seeland, Germany / Center for Integrated Breeding Research (CiBreed), Georg-August-University, Göttingen, Germany

Hordeum bulbosum is the closest wild relative of barley (Hordeum vulgare). Wide crosses between both species have been used to obtain doubled haploid progeny and develop disease-resistant barley introgression lines. The H. bulbosum genome is highly heterozygous and, in some genotypes, autotetraploid. Advances in genome assembly methodology, notably PacBio accurate-long-read (HiFi) and chromosome conformation capture sequencing (Hi-C), have greatly expanded our ability to assemble and phase heterozygous and autopolyploid genomes.
Here, we report on the construction of diploid and tetraploid H. bulbosum genome sequence assemblies using the TRITEX computational pipeline. We validated the assembly of one diploid clone by genetic mapping and fluorescence in situ hybridization of haplotype-specific chromosome painting probes. Diversity between H. bulbosum haplotypes was higher than between those of H. vulgare. Divergence between H. bulbosum and H. vulgare was variable along the genome. Genetic recombination was confined to distal chromosomal regions in H. bulbosum to an even greater extent than in barley. These genomic resources will further our understanding of variation between diploid and tetraploid cytotypes of H. bulbosum and the characterization of barley crop-wild introgression lines.

KEYWORDS: Hordeum bulbosum, genome, haplotype, barley

IDENTIFICATION OF GENES INVOLVED IN SPIKE ARCHITECTURE AND TIME OF FLOWERING IN BARLEY

Marek Marzec
Faculty of Natural Sciences, University of Silesia, Katowice, Poland
marek.marzec@us.edu.pl

Co-Author:

Agnieszka Janiak
Faculty of Natural Sciences, University of Silesia, Katowice, Poland

TILLING (Targeting Induced Local Lesions IN Genomes) is a reverse genetics strategy that identifies mutations in specific genes of interest in chemically mutagenized populations. However, the TILLING population can also be used in the forward genetics approach if an interesting phenotype is found among plants after mutagenesis. Here, we present two barley mutants from the HorTILLUS (Hordeum-TILLING-University of Silesia) population, created for spring barley cultivar Sebastian after double-treatment of seeds with two chemical mutagens: sodium azide (NaN3) and N-methyl-N-nitrosourea (MNU). Both mutants were selected based on the phenotype, different from parental variety. The first mutant called hercules, exhibited intermediate spikes, whereas wild-type Sebastian is a two-row spike cultivar. The second mutant, speedy, reaches maturity three weeks earlier than the parent variety. Crossing of mutants with wild-type revealed that the single, recessive allele is responsible for phenotypes in both cases. Whole-genome sequencing of individuals from F2 populations with mutant and wild-type phenotypes was performed to identify mutated genes using Bulk Segregant Analysis approach. WGS data indicate a set of candidate genes carried SNPs, which may be responsible for hercules and speedy phenotype. Those candidate genes are the subject of co-segregation analysis toward the identification of loci responsible for spike development and time of flowering, respectively.

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KEYWORDS: flowering time, mutagenesis, spike development, TILLING, whole genome sequencing

GENETIC AND PHYSICAL LOCALIZATION OF A SPOT FORM NET BLOTCH SUSCEPTIBILITY GENE IN BARLEY

Shengming Yang
USDA-ARS Cereals Research Unit, Edward T. Schafer Agriculture Research Center, Fargo, ND 58102, USA
Shengming.Yang@usda.gov

Co-Authors:

Abdullah Fahad Alhashel, Thomas Baldwin
Department of Plant Pathology, North Dakota State University, Fargo, ND 58102, USA

Caused by the necrotrophic fungal pathogen Pyrenophora teres f. maculata (Ptm), spot form net blotch (SFNB) is one of the important foliar diseases in barley. Although various resistance loci have been identified, breeding for SFNB-resistant varieties has been hampered due to the complex virulence profile of Ptm populations. One resistance locus in the host may be effective against one specific isolate, but it may be susceptible to other isolates. However, a major susceptibility QTL on 7H, named Sptm1, was consistently identified in many studies. To fine map towards cloning of the Sptme1 gene, we conduct genetic mapping in the present study. A segregating population was developed from selected F2s of Tradition (S) x PI67538 (R), in which the disease phenotype was determined by the Sptm1 locus alone. Gene prediction and annotation identified five protein-coding genes in the Sptm1 region. Genome resequencing revealed single nucleotide polymorphisms (SNPs) between alleles for one gene only, which encodes a putative cold responsive protein kinase. Therefore, providing fine localization and candidate of Sptm1 for functional validation, our study will facilitate the understanding of susceptibility mechanism underlying the Ptm-barley interaction and offer potential target for gene editing to develop valuable material with broad-spectrum resistance to SFNB.

Aknownledgements: North Dakoda Barley Council

KEYWORDS: Spot form net blotch, barley, genetic mapping, disease resistance

A CONSOLIDATED GENE ANNOTATION FOR THE BARLEY PAN-GENOME

Thomas Lux
Plant Genome and Systems Biology, Helmholtz Center Munich, German Research Center for Environmental Health (GmbH)

thomas.lux@helmholtz-muenchen.de

Co-Authors:

Heidrun Gundlach, Klaus F.X. Mayer, Manuel Spannagl
Plant Genome and Systems Biology, Helmholtz Center Munich, German Research Center for Environmental Health (GmbH)

Martin Mascher, Nils Stein
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)

International Barley Pangenome Consortium

Accurate and complete structural gene annotation is essential for any pan-genome study, especially for large and highly repetitive genomes like the barley genome. Erroneous gene annotations will lead to false or incomplete conclusions. Here we present an enhanced protocol developed for the barley pan-genome project to unify individual gene predictions from all sequenced genotypes.
Based on the already established annotation process published as part of the H. vulgare Morex reference genome paper additional methods were refined to counteract possible annotation errors. To aid the initial structural annotation RNAseq data derived from different tissues and developmental stages, as well as ISOseq data were generated for each barley accession. Combined with protein homology and ab initio gene predictions we were able to define accurate gene models for each genotype.
A supplementary consolidation step that overcomes fuzzy limitations in gene calling on individual genomes ensures a uniform gene annotation across all genomes. This consolidation method is based on whole genome alignments to identify syntenic regions, cross-map gene models of each of the lines and rectify wrong or missing gene models. Along with the availability of reference-quality genome sequences for 20 genetically diverse barley genotypes unified gene annotations will leverage any further downstream genomic and breeding-related analyses.

KEYWORDS: barley, pangenome, annotation

BIOTIC STRESSES UNDER CLIMATE CHANGE

PAN-GENOME ENABLED DISEASE RESISTANCE GENE DISCOVERY IN WILD BARLEY

Brian J. Steffenson
Department of Plant Pathology, University of Minnesota, Saint Paul, MN USA
bsteffen@umn.edu

Co-Authors:

M. Mascher, M. Jayakodi, A. Himmelbach, U. Scholz, N. Stein
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

G. Bethke, G. J. Muehlbauer
Department of Agronomy and Plant Genetics, University of Minnesota, Saint Paul, MN USA

A. Sallam, O. Matny
Department of Plant Pathology, University of Minnesota, Saint Paul, MN USA

I. Mayrose, L. Glick
School of Plant Sciences and Food Security, Tel Aviv University, Tel Aviv, Israel

Cultivated barley is vulnerable to many diseases across different production regions of the world. The deployment of resistant cultivars is the most effective and economically sound approach for disease control. In breeding for resistance, it is important to have a pool of diverse resistance genes that can be combined to provide a wider spectrum of resistance and greater durability. Wild barley (Hordeum vulgare ssp. spontaneum) is the progenitor of cultivated barley and is known as a rich source of disease resistance genes. To exploit this taxon for new resistance genes useful in barley breeding, a diverse collection of accessions was assembled across its distribution range.

This Wild Barley Diversity Collection (WBDC) consists of 314 accessions selected based on various ecogeographic criteria. Most of the accessions were collected from the Fertile Crescent (77.4%), but samples were also included from Central Asia (15.8%), North Africa (3.8%), and the Caucasus region (2.8%). Extensive disease phenotyping studies were conducted on seedlings of the WBDC to isolates of the powdery mildew, stem rust, leaf rust, stripe rust, net blotch, spot blotch, and leaf scald pathogens. Initial genome-wide association studies (GWAS) using single nucleotide polymorphism markers generated by genotyping-by-sequencing revealed new resistance loci for the different diseases.

To clone multiple nucleotide binding site-leucine rich repeat (NLR) type resistance genes, a genome complexity reduction protocol (Association Genetics Resistance Enrichment Sequencing or AgRenSeq) was applied to the WBDC and led to the cloning of four strong candidate genes for resistance to powdery mildew and two for resistance to leaf rust. Although AgRenSeq facilitated the identification and cloning of some NLR genes in wild barley, we encountered several difficulties that hindered our ability to readily capture other genes. The most critical difficulty was that contigs identified by AgRenSeq contained very small portions of the promoter sequences for the resistance genes and therefore required a time-consuming genome walking approach to capture this region.

To overcome this difficulty and also capture non-NLR resistance genes, the WBDC was sequenced at 10x depth through the International Wild Barley Sequencing Consortium (https://iwbsc.umn.edu/). Preliminary GWAS using whole genome sequence data revealed many highly significant signals for both previously mapped resistance loci and many new resistance loci. Pangenome-informed GWAS is now underway using the cultivated and wild barley pangenomes.

This analysis will enable us to accurately determine the genomic positions of resistance genes and distinguish novel alleles in wild barley that are not present in cultivated germplasm. The resulting information will facilitate the efficient usage of novel resistance genes from wild barley for cultivated barley improvement.

Aknownledgements: We acknowledge the contributions of the International Wild Barley Sequencing Consortium

KEYWORDS: wild barley, disease resistance, genetic resources, whole genome sequencing

RPT5 ENCODES A RECEPTOR-LIKE PROTEIN THAT PROVIDES THE BROADEST AND MOST EFFECTIVE NET FORM NET BLOTCH (PYRENOPHORA TERES F. TERES) RESISTANCE IN BARLEY

Karl Effertz
Department of Crop and Soil Sciences, Washington State University, Pullman, WA, USA
karl.effertz@wsu.edu

Co-Authors:

Jon Richards
Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA, USA

Shaun Clare, Madeline Del Castillo, Robert Brueggeman
Department of Crop and Soil Sciences, Washington State University, Pullman, WA, USA

Roshan Sharma Poudel
Sygenta Seed Inc., Durham, NC 27709, USA

Matthew Moscou
The Sainsbury Laboratory, University of East Anglia, Norwich, England, UK

Timothy Friesen
Cereal Crops Research Unit, Northern Crop Science Laboratory, USDA-ARS, Fargo, ND, USA

Net form net blotch (NFNB), caused by the necrotrophic fungal pathogen Pyrenophora teres f. teres (Ptt), causes yield loss of barley worldwide. Resistance conferred by the Rpt5 locus from barley line CI5791 is the broadest resistance reported in this pathosystem. However, the dominant susceptibility gene (Spt1) also present at this locus in other lines lends to the complexity of this centromeric region of chromosome 6H. The Rpt5/Spt1 locus was reported in many studies investigating Ptt resistance and susceptibility and appears to contain multiple alleles of Rpt5 and Spt1.
Our high-resolution mapping revealed that double recombination occurs surrounding the locus in approximately one percent of recombinant gametes with no individuals harboring recombination within the ~2 Mb region delimited by these double recombination events. The phenomenon was observed in a CI5791 (R) x Tifang (S) F2 population, a CI5791 x Tifang recombinant inbred line population and a Steptoe x Morex doubled haploid population, leading us to hypothesize a conserved evolutionary mechanism to retain the function of this important locus. Candidate genes within the delimited region also harbor unprecedented levels of polymorphism, suggesting pathogen pressure is driving diversification as many Ptt effectors target the region. Two Rpt5/Spt1 candidate genes were identified and Golden Promise transformants containing the Rpt5/Spt1 candidate gene 1 (Rcg1) allele from line CI5791 showed a significant shift towards resistance. We present genetic and functional validation data supporting the identification of the Rpt5/Spt1 gene which begins to uncover the complex molecular mechanisms underlying this resistance providing insight into broad necrotrophic resistance.

KEYWORDS: Necrotroph, Pathogen, Disease Resistance, Co-Evolution

FUNCTIONAL DIVERSIFICATION OF A BARLEY RECEPTOR KINASE INVOLVED IN IMMUNITY TO WHEAT STRIPE RUST

Molly Bergum
The Sainsbury Laboratory, Norwich, UK
Molly.bergum@tsl.ac.uk

Co-Authors:

Samuel Holden, Anupriya Thind, Jack Rhodes, Inmaculada Hernández-Pinzón, Phon Green, Matthew Moscou
The Sainsbury Laboratory, Norwich, UK

Silvio Salvi
Università di Bologna, Bologna, Italy

Christine Faulkner
John Innes Centre, Norwich, UK

Pattern triggered immunity mediated by receptor kinases (RKs) is an important aspect of the plant immune system and serves as a source of resistance to the devastating wheat stripe rust pathogen in barley. In plant-pathogen interactions, three RKs have been extensively studied: FLS2 in angiosperms, EFR in Brassicaceae, and Xa21 in rice. These RKs provide bacterial resistance and belong to the LRR-XII subfamily. We identified and cloned an LRR-XII member in barley: Puccinia striiformis receptor kinase1 (Pur1), which is the homolog of Xa21. Pur1 belongs to the Rps8 locus, which is a 936 kb region that encompasses a 546 kb presence-absence variation in diverse barley accessions and confers resistance to the non-adapted fungal pathogen Puccinia striiformis f. sp. tritici. Using transcriptomics, a forward genetic screen, and transgenic complementation, we found that Rps8-mediated resistance requires a genetic module of two genes within this locus: Pur1 and Exo70FX12. Exo70 proteins are canonically involved in the exocyst complex, although phylogenetic evidence indicates that Exo70FX12 belongs to a Poales-specific clade with unknown function. We hypothesized that the requirement of Exo70FX12 may suggest lineage-specific requirements for Pur1 function. Previous work has shown that the Xa21 kinase domain is functional in Nicotiana benthamiana when fused to the ectodomain of EFR. To test whether the Pur1 kinase domain can function in this heterologous system, we generated chimeric constructs with the EFR extracellular domain and transmembrane/intracellular domains of Xa21 or Pur1 fused with a C-terminal GFP tag. We transiently expressed constructs in N. benthamiana and exposed leaves to the ligand of EFR, elf18. The Pur1 kinase domain is unable to induce a burst of reactive oxygen species (ROS), unlike the kinase domain of Xa21, which retains function. Co-expression of Exo70FX12 with the EFR-Pur1-GFP construct did not confer gain of function in this heterologous system, indicating the likely requirement of additional monocot-specific elements.
Pur1 induces resistance in barley to a fungal pathogen and requires at least one lineage-specific gene, suggesting that it has functionally diverged from FLS2, EFR, and its ortholog Xa21. It remains unclear if the dependency of Pur1 on Exo70FX12 is rooted in RK secretion or signal transduction. Future work to understand this source of rust immunity in barley involves elucidating the mechanistic relationship between Pur1 and Exo70FX12 and uncovering additional genes involved in Rps8-mediated resistance.

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KEYWORDS: LRR-XII subfamily, Exo70, nonhost resistance, Puccinia striiformis

CONVERGENT MECHANISM OF HOST SUSCEPTIBILITY FACTORS ASSISTING GENETIC IMPROVEMENT OF THE BYMOVIRUS RESISTANCE IN BARLEY AND WHEAT

Ping Yang
Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China
yangping@caas.cn

Co-Authors:

Jinhong Kan, Yu Cai, Chunyuan Cheng, Congcong Jiang, Yanlong Jin
Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China

Plant viruses account for almost 50% of the pathogens responsible for emerging plant diseases worldwide. Plant RNA viruses have relatively small genomes and encode very limited proteins, which lead to a need of host cell factors to complete their life cycles. Modifications of these host factors could block the susceptibility, causing recessive resistance. Bymoviruses transmitted by the soil-borne plasmodiophorid Polymyxa graminis severely threaten globally important cereal crops, including barley and wheat.
The bymovirus barley yellow mosaic virus (BaYMV) and barley mild mosaic virus (BaMMV) cause barley yellow mosaic disease, and the wheat yellow mosaic virus (WYMV) causes wheat yellow mosaic disease. In cultivated barley, two host factors protein disulphide isomerase like 5-1 (PDIL5-1) and eukaryotic translation initiation factor 4E (eIF4E) to BaYMV/BaMMV been identified, whereas none of host factors to WYMV has been identified in hexaploid wheat.
To test if the host factor accounting for recessive resistance was blinded by functional redundancy of the homoeoalleles in polyploid species, the genome editing technology with CRISPR/Cas9 machinery was applied to simultaneously knockout the three homoeoalleles of barley HvPDIL5-1 and HveIF4E homologous genes in wheat, respectively. Single, double, and triple knockout mutants of TaPDIL5-1 or TaeIF4E were obtained through cross-pollination and marker-assisted selection. By inoculating with WYMV, the single- and double-mutants were susceptible as the same as wild-type plants, while the triple-mutants showed complete resistance in either TaPDIL5-1 or TaeIF4E edited lines.
We further investigated the agronomic performance of these mutants and wild-type plants. No yield penalty in the TaPDIL5-1 edited lines was observed as the same as that in cultivated barley, while editing of the TaeIF4E homoeoalleles causes pleiotropic effects is under investigation. Collectively, we demonstrated an efficient strategy in deciphering virus resistance in Triticeae crops, such as identifying the host factors in diploid barley, followed by genome editing of their homoeoalleles in polyploid species.

KEYWORDS: Barley, Wheat, Bymovirus, Host resistance, Genome editing

RYD4HB: A MAJOR RESISTANCE LOCUS TO BYDV FROM THE BARLEY WILD RELATIVE HORDEUM BULBOSUM WITH A VERY LARGE DIVERSITY IN BARLEY GERMPLASM

Hélène Pidon
Julius Kühn-Institute (JKI), Institute for Resistance Research and Stress Tolerance, Quedlinburg, Germany; Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Seeland OT Gatersleben, Germany
pidon@ipk-gatersleben.de

Co-Authors:

Neele Wendler, Viktor Korzun
KWS SAAT SE & Co. KGaA, Einbeck, Germany

Antje Habekuß
Julius Kühn-Institute (JKI), Institute for Resistance Research and Stress Tolerance, Quedlinburg, Germany

Klaus Oldach
KWS LOCHOW GmbH, Bergen, Germany

Murukarthick Jayakodi, Robert Hoffie, Jochen Kumlehn, Martin Mascher
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Seeland OT Gatersleben, Germany

Frank Ordon
Julius Kühn-Institute (JKI), Quedlinburg, Germany

Nils Stein
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Seeland OT Gatersleben, Germany ; CiBreed - Center for Integrated Breeding Research, Georg-August University, Göttingen, Germany

Virus diseases are causing high yield losses in crops worldwide. In this respect, the Barley yellow dwarf virus (BYDV) complex is responsible for one of the most widespread and economically important viral diseases of cereals. Transmitted by aphids, its incidence in the fields in Europe is increasing; associated with the reduction of insecticide use and led by the increasingly warm autumns, driving more frequent aphid infestations. To achieve durable protection of the crop, the use of resistance genes appears as one of the best tools. While no complete resistance gene has been uncovered in the primary genepool of barley (Hordeum vulgare), one locus providing a dominant resistance to BYDV was identified in the wild relative Hordeum bulbosum. Intraspecific crosses were performed, resulting in a resistant introgression line carrying a single subterminal H. bulbosum introgression on chromosome 3HL (Scholz et al, Theor Appl Genet. 2009) We are aiming at cloning this locus, named Ryd4Hb. Recombination events in crosses between H. vulgare and H. bulbosum are scarce and the interval identified in a population of a thousand plants was large and imprecise. Taking advantage of high-throughput genotyping methods, we screened a population of 16,000 F2 plants, in which we identified less than 120 recombinant plants in a 13.3 Mbp interval. Ultimately, we were able to reduce the interval to 67 kbp on the barley reference genome, annotated with two genes from the nucleotide-binding and leucine-rich repeat immune receptors family (NLR), including a pseudogene. However, this locus presents a very large structural diversity in the recently published barley pangenome, including a large number of duplications of the candidate NLR. To identify the candidate genes in the resistant plant, we assembled the genome of the resistance donor introgression line, revealing a 700 kbp-long interval in which four complete NLRs were identified. Functional validation of those candidates is ongoing using C as9 endonuclease-mediated knock-outs in the introgression line.

KEYWORDS: Virus, resistance, barley, BYDV

COLLABORATIVE APPROACH FOR BIOTIC STRESSES PHENOTYPING EXCELLENCE IN DEVELOPING WORLD

Ramesh Pal Singh Verma
Indian Institute of Wheat and Barley Research, Karnal-132001, India (Formerly Barley Breeder, ICARDA Morocco)
rpsvdwr@hotmail.com

Co-Authors:

Sajid Rehman, Sanjaya Gyawali
IICARDA, Morocco (Formerly ICARDA)

Girma Fana
ARS Sinana, Ethiopia

Safaa G Kumari, SC Bhardwaj
IIWBR India

Berhane Lakew
EIAR, Holetta, Ethiopia

Barley is an ancient cereal crop with a significant role in the human civilization. Its major end-uses uses across the globe include feed, and fodder for animals and health food for mankind, besides for malting and brewing industries. Under the fast-changing climate, it is considered as a future crop of choice as this can thrive well under the diverse situations. This also includes the varying biotic stresses incidences with changed climates across the regions. The developing world heavily depends for access to elite germplasm on CGIAR, where ICARDA plays the main role for germplasm improvement incorporating the tolerance to biotic and abiotic stresses, nutritional and industrial quality and desired agronomic traits required for highly diverse target environments. Exploration for genetic diversity and trait discovery is always suggested for further improvement of barley to match barley breeding progress with the climate changes. In order to conduct a successful trait discovery in genetic resources and then formulate regional breeding strategies to combat the challenges posed by diseases and pests, a collaborative phenotyping strategy was followed involving ICARDA network with partner countries in North Africa (Morocco, Tunisia and Lebanon), East Africa (Ethiopia) and South Asia (India).

A common set of 316 genotypes across feed and malting types originating from different continents consisting of 161 cultivars, 21 landraces, 134 advanced breeding lines, representing two-row (173) and six-row (143) types was genotyped with 50K SNPs and used in the study. Phenotyping of for a number of fungal diseases Viz. Scald, net blotch, spot blotch, powdery mildew, stripe and leaf rusts was conducted from 2018 to 2020 at field under hotspot / artificial epiphytotic conditions with locally prevalent pathotypes/ races. The seedling screening under controlled conditions for stripe and leaf rusts against 7 pathotypes in India and net blotch screening against most prevalent pathotypes in Morocco has been completed. Similarly, the same set has been phenotyped for tolerance to barley yellow dwarf Virus-PAV under artificial inoculation in Tunisia and Ethiopia.

The results of the evaluation are very encouraging with a number of resistant/ tolerant genotypes have been recorded for each disease including the seedling screening with specific pathotypes. Based on their geographic origins it is assumed that a lot of diversity might be in hand for the resistances. Similarly, for BYD-PAV also we have observed several lines each in Tunisia and Ethiopia with very effective tolerance levels. The GWAS studies for individual disease/ locations are underway with the genotypic data already available. The information generated will be of great importance in global barley resistance breeding programme for biotic stresses as barley is low input crop for small holder farmers in the developing world where chemical control is not common.

Aknownledgements: All partner organizations and CRP Dryland Cereals for supporting the study

KEYWORDS: Barley, Genetic improvement, Biotic stresses, phenotyping, climate change

GENOMIC AND PATHOGENIC DIVERSITY OF BARLEY YELLOW MOSAIC VIRUS AND BARLEY MILD MOSAIC VIRUS ISOLATES IN FIELDS OF CHINA AND THEIR COMPATIBILITY WITH RESISTANCE GENES OF CULTIVATED BARLEY

Ping Yang
Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China
yangping@caas.cn

Co-Authors:

Congcong Jiang, Shenghui Zhou, Yu Cai, Jing Zhang
Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China

Miaomiao Lei, Zongyun Feng
College of Agronomy, Sichuan Agricultural University, Chengdu, China

Haiye Luan, Xiao Xu, Huiquan Shen
Jiangsu Institute for Seaside Agricultural Sciences and Yancheng Academy of Agricultural Science, Yancheng, China

Yuhan Pan, Rugen Xu
College of Agronomy, Yangzhou University, Yangzhou, China

Li Zhang
College of Life Sciences, Tsinghua University, Beijing, China

Plant viruses transmitted by the soil-borne plasmodiophorid Polymyxa graminis constantly threaten global production of cereal crops. Although the yellow mosaic virus disease of barley has been known to be present for a long time in China, the understanding of the diversity of the viral pathogens and their interactions with host resistance remains limited. In this study, we conducted a nationwide survey of P. graminis and the barley yellow mosaic virus (BaYMV) and barley mild mosaic virus (BaMMV) it transmits, followed by genomic and pathogenic diversity analyses of both viruses. BaYMV and BaMMV were found exclusively in the region downstream of the Yangtze River, despite the national distribution of its transmission vector P. graminis.

Analysis of the genomic variations of BaYMV and BaMMV revealed an elevated rate of non-synonymous substitutions in the viral genome-linked protein (VPg), in which most substitutions were located in its interaction surface with the host eukaryotic translation initiation factor 4E (eIF4E). VPg sequence diversity was associated with the divergence in virus pathogenicity that was identified through multiple field trials. The majority of the resistance genes, including the widely-applied rym4 and rym5 (alleles of eIF4E), as well as the combination of rym1/11 and rym5, are not sufficient to protect cultivated barley against both viruses in China. Collectively, these results provide insights into virulence specificity and interaction mode with host resistance in cultivated barley, which has significant implications in breeding for the broad-spectrum resistance barley varieties.

KEYWORDS: Barley, BaYMV/BaMMV, Host resistance, Pathogenicity, rym gene

DEVELOPMENT OF A MARKER WITHIN THE CANDIDATE UN8 TRUE LOOSE SMUT RESISTANCE GENE FOR USE IN LATVIAN BARLEY BREEDING

Elina Sokolova
Priekuli Research Centre, Institute of Agricultural Resources and Economics, 2 Zinatnes st., Priekuli, LV4126, Latvia

elina.sokolova@arei.lv

Co-Authors:

Šķipars V., Ruņģis D.
Genetic Resource Centre, Latvian State Forest Research Institute "Silava", 111 Rigas st. , Salaspils, LV2169, Latvia

Seile S., Legzdiņa L.
Priekuli Research Centre, Institute of Agricultural Resources and Economics, 2 Zinatnes st., Priekuli, LV4126, Latvia

Breeding for resistance to true loose smut infection caused by the pathogen (Ustilago nuda (Jens.) Rostr.) can be an economical and environmentally safe way to limit the effect of this pathogen on barley and is especially essential while breeding for organic agriculture where chemical treatment of seed cannot be applied. However, screening for resistance using natural infection can lead to inconsistent results and artificial inoculation is labour intensive and not always successful. Thus marker assisted selection using genetic markers closely linked with alleles conferring disease resistance, can increase the efficiency of breeding programs. A candidate gene for Un8 resistance was used to develop a genetic marker, which was tested on a barley recombinant inbred line (RIL) population created in Latvia. The RIL parental varieties were the resistant 'CDC Freedom' and the susceptible 'Samson'. The F5 RIL population consisted of 98 lines, which were phenotyped for resistance to true loose smut by both natural infections and artificial inoculations. The candidate Un8 gene was sequenced in both parental varieties. A PvuII restriction enzyme site was identified in the resistant 'CDC Freedom' variety, which was absent in the susceptible 'Samson' variety. PCR primers were designed to amplify a 170 base pair region flanking this site. Genotyping of the 98 lines from the RIL population with the new marker Un8-PvuII showed a 99% correspondence with artificial inoculation results. The restriction marker was further tested and validated in other barley varieties and breeding lines.

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KEYWORDS: marker-assisted breeding, pathogen resistance, resistance breeding

HVOZ.26 - A NEWLY IDENTIFIED RECEPTO CENTRAL FOR PLANT ABIOTIC AND BIOTIC STRESS TOLERANCE

Triin Vahisalu
University of Helsinki
triin.vahisalu@helsinki.fi

Understanding plant response mechanisms to biotic and abiotic stresses at the molecular level is essential for developing cultivars that perform better in a shifting global climate. Biotic and abiotic stress perception leads to reactive oxygen species (ROS) production, which activates tightly coordinated and regulated defense pathways (Kimura et al., 2017).
Molecular mechanisms in stress signaling activation have been studied extensively in Arabidoposis and numerous components characterized at the molecular level. Nevertheless, relevant signaling pathways are still incomplete and need further discoveries (Foyer and Noctor 2016).
From a large-scale mutant screen based on ozone (O3) sensitivity in Arabidopsis, I have identified and mapped a novel component – OZ.26, being involved in early biotic and abiotic stress responses, ubiquitously expressed in various plant tissues and localized to the ER. oz.26 mutants are highly sensitive to O3, Pseudomonas, Botrytis and freezing but not to drought, salt and heat tratments, showing specificity. Additionally, oz.26 mutants show elevated ROS levels in response to flg22 and chitin treatments when compared to WT. Furthermore, I have shown that O3 and flg22 induce autophagy in OZ.26-YFP lines. Autophagy is a highly conserved major degradation and recycling pathway in plants, an interplay between ROS and autophagy has been observed previously. NADPH dependent ROS activate autophagy to degrade damaged organelles and oxidized proteins rapidly and effectively in plant cells (Tang et al., 2018).
OZ.26 carries a putative heme binding domain. Heme plays an active role in plant metabolic pathways as well as in stress signaling. Oxidative stress leads to increased heme content in the cell (Vanhee et al., 2011) and accumulating free heme molecules are capable of reacting with oxygen to generate cytotoxic ROS (Busch and Montgomery, 2015). Recently I have shown that OZ.26 has heme binding properties by applying Microscale Thermophoresis (MST).
The function of heme and heme receptors in stress signaling in crops has not been addressed so far. With a translational biology approach I'm addressing the function of OZ.26 in barley. I have been able to identify and isolate the barley homolog of OZ.26. Relevant Arabidopsis and barley protein sequences are highly conserved, putative regulatory amino acids being 100% conserved. Currently CRISPR/Cas9 edited HvOZ.26 lines are being made in order to address the function in barley. Heme binding properties of HvOZ.26 will be addressed in parallel with general phenotyping.
Thus, OZ.26 is a novel heme receptor protein, essential for immune responses in plants. With sufficient evidence, OZ.26 could be a potential target of manipulation that may lead to agronomic benefits and used as an input to precision breeding in the future.

KEYWORDS: translational biology, biotic stress tolerance, abiotic stress, receptor proteins

HOST SPECIFICITY OF SOIL-BORNE PATHOGENS IN HORDEUM SPECIES AND THEIR RELATIVES

Ping Yang
Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China
yangping@caas.cn

Co-Authors:

Yanlong Jin, Congcong Jiang
Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China

Shiqiang Chen, Zhentian He
Lixiahe Institute of Agriculture Sciences in Jiangsu Province, Yangzhou, China

Xiao Xu, Huiquan Shen
Jiangsu Institute for Seaside Agricultural Sciences and Yancheng Academy of Agricultural Science, Yancheng, China

Wanquan Ji
State Key Laboratory of Crop Stress Biology for Arid Areas, College of Agronomy, Northwest A&F University, Yangling, China

Soil-borne pathogens destabilize the yields of Triticeae crops, including barley (Hordeum vulgare L.) and wheat (Triticum aestivum L.). Although genetic resistance derived from relatives of these species has been utilized to prevent rust diseases (i.e. ithe wheat-rye 1BL-1RS translocation line), research on resistance against soil-borne pathogens remains limited. Here, we performed field trials using 76 genotypes representing 28 Hordeum, six Triticum, and two Aegilops species to examine resistance against three soil-borne bymoviruses: barley yellow mosaic virus (BaYMV), barley mild mosaic virus (BaMMV), and wheat yellow mosaic virus (WYMV). We also performed greenhouse tests using the soil-borne fungal pathogen Fusarium pseudograminearum, which causes Fusarium crown rot (FCR). Using RT-PCR, we detected BaMMV and BaYMV in several Hordeum species, whereas WYMV set up systemic infection in the Triticum and Aegilops species.
The identification of FCR susceptibility in all species examined suggests that F. pseudograminearum is a facultative fungal pathogen in Triticeae. Intra-species variation in FCR disease severity was observed for several species, suggesting the possibility of exploring host resistance mechanisms. Therefore, by unlocking the host specificity of four soil-borne pathogens in Hordeum species and their relatives, we obtained insights for the further exploration of wild sources of soil-borne pathogen resistance for future wheat and barley improvement programs.

KEYWORDS: Hordeum, Soil-borne, Bymovirus, F. pseudograminearum, Fusarium crown rot (FCR)

TOWARDS DECIPHERING VARIOUS BARLEY LEAF RUST RESISTANCES IN THE MBR1012 X SCARLETT POPULATION

Yu Cai
Julius Kuehn-Institute (JKI), Federal Research Institute for Cultivated Plants, Institute for Resistance Research and Stress Tolerance, 06484, Quedlinburg, Germany

yu.cai@julius-kuehn.de

Co-Authors:

Davinder Singh, Peter Dracatos, Robert F. Park
Plant Breeding Institute, The University of Sydney, Private Bag 4011, Narellan, NSW 2567, Australia

Jens Keilwagen, Frank Ordon, Dragan Perovic
Julius Kuehn-Institute (JKI), Federal Research Institute for Cultivated Plants, Institute for Resistance Research and Stress Tolerance, 06484, Quedlinburg, Germany

The rapid evolution of rust pathogens frequently leads to outbreaks of rust diseases that are known to cause high yield losses in cereals. Success in breeding rust resistant barley cultivars is challenged by the high ability of rust pathogens to overcome resistance genes. Therefore, discovering and deploying novel resistance genes/alleles is the most economical and environmentally friendly approach to combat disease. Here, we evaluated the genetic basis of seedling leaf rust resistance and adult plant resistance (APR) across contrasting environments in Australia and Germany.

The genetic basis of leaf rust resistance in barley landrace MBR1012 was evaluated at the seedling stage by phenotyping a mapping population comprising 97 doubled haploid (DH) lines of the cross MBR1012 x Scarlett (M/S) with the highly virulent P. hordei race I80 from Europe and several Australian races with contrasting pathogenicity. The M/S DH population was genotyped using the 15K iSelect chip platform. A single dominant gene (RphMBR1012) against race I80 on chromosome 1HS was identified. This gene locus was subsequently mapped to a 0.05 cM interval, corresponding to a physical region of 433 Kb in the reference genome MorexV3.

The population was also phenotyped in Australia in seedling tests with five diverse Australian P. hordei races (5457 P+, 5652 P+, 253 P-, 220 P+ Rph13+ and 200 P-) and under field conditions in Australia over two seasons using race 5457 P+, permitting genetic analysis and the identification of adult plant resistance (APR). For APR, a single QTL was mapped on chromosome 2HL in both years, explaining 17.4% and 21.1% of the phenotypic variance in 2019 and 2020, respectively. This QTL might be a novel APR locus originating from MBR1012. In addition to the APR on 2HL and RphMBR1012, two minor loci on chromosome 5HS and a significant locus on chromosome 7HL in response to all isolates except race 5457P+ were identified. Among them, locus on chromosome 7H could explain the phenotypic variance from 13.6% to 49.2%, while loci on 5H could provide only 11.1% to 18.5% of phenotypic variance. These QTLs were found to be overlapped with Rph2 on chromosome 2HS and Rph3 on chromosome 7HL, respectively. Of these, resistance loci on chromosome 1H and 7H for seedling stage resistance and APR locus on chromosome could be used for genetic improvement in barley.

KEYWORDS: cultivated barley, genetic mapping, Puccinia hordei, adult-plant resistance (APR)

GENOME WIDE ASSOCIATION MAPPING FOR NET FORM OF NET BLOTCH IN THE ICARDA'S HI-AM PANEL REVEALS GENOMIC HOTSPOTS FOR BARLEY MULTIPLE RESISTANCE TO BIOTROPHIC AND NECROTROPHIC DISEASES IN BARLEY

Andrea Visioni
Biodiversity and Crop Improvement Program, International center for Agricultural Research in the Dry Area (ICARDA). Rabat, Morocco

a.visioni@cgiar.org

Co-Authors:

Sajid Rehman
Field Crop Development Center, Olds College, Lacombe, Canada

Ramesh Pal Singh Verma
Indian Institute of Wheat and Barley Research, Karnal-132001, India

Sanjaya Gyawali
Sakata Seed America Inc., 1857 Bay Ridge Dr, Burlington, WA 98233 USA

Ayed M. Al-Abdallat
Department of Horticulture and Crop Science, the faculty of Agriculture, University of Jordan

Miguel Sanchez-Garcia
Biodiversity and Crop Improvement Program, International center for Agricultural Research in the Dry Area (ICARDA). Rabat, Morocco

The net form net blotch (NFNB) caused by Pyrenophora teres f. teres (Ptt), is an emerging barley disease in several countries. The foliar infection causes severe yield losses while the infection of other aerial structures such as leaves, kernels and stems also impact yield and quality. Although NFNB can be effectively managed with fungicides, the use of resistant varieties is considered the most sustainable option for both environmental and economic reasons. Incorporation of qualitative and quantitative resistance is important to obtain cultivars with durable resistance to NFNB. For this purpose, an association mapping panel named as HI-AM (High Input Association Mapping) was screened for resistance to NFNB at seedling stage with two virulent Moroccan isolates under controlled conditions, and at the adult plant stages at three hot spot locations in Morocco during different cropping seasons (2013-14, 2015-16 and 2017-18). About 4 genotypes showed combined resistance to both Ptt isolates tested at seedling stage, while 27 genotypes were resistant to moderately resistant at adult plant stage at all field locations tested. HI-AM encloses 261 genotypes of different origins selected to represent different end uses under optimum management conditions. Out of 261 entries, 124 were from ICARDA's barley breeding program (50 two-row and 74 six-row type), 32 from Europe (28 two-row and 4 six-row type), 34 North America (28 two-row and 6 six-row type), 67 from South America (62 two-row and 5 six-row type) and 4 from Australia (only two-row type). The genome wide association mapping (GWAM) was conducted in TASSEL (v 5.0) using 13,182 PAV and 6,311 SNP markers and phenotypic data. Results of GWAM showed 7 QTL for resistance to Ptt isolate Ptt40-3, and 11 QTL for Ptt45-3. A total of 38 QTL were associated with the adult plant stage resistance. Interestingly several QTL detected in this study were located in the same genomic regions where QTL were detected previously for resistance to stripe rust and spot blotch in the same panel, highlighting the presence of genomic hotspots for multiple disease resistance (MDR) on barley genome.

MDR have also been reported in wheat, arabidopsis, maize and rice. The identification of germplasm with different origin and end use carrying the beneficial alleles at the MDR loci could be of great help for breeders in developing new varieties with enhanced resistance to multiple diseases.

Aknownledgements: This research was funded by CGIAR Research Program (CRP) on Dryland Systems.

KEYWORDS: Net blotch, Moroccan isolates, GWAS, Multi Disease Resistance Loci

ABIOTIC STRESSES UNDER CLIMATE CHANGE

CELL-BASED PHENOTYPING APPROACH FOR IMPROVING ABIOTIC STRESS TOLERANCE IN BARLEY

Sergey Shabala
Tasmanian Institute for Agriculture, University of Tasmania, Australia; International Research Centre for Environmental Membrane Biology, Foshan University, China
sergey.shabala@utas.edu.au

To match predicted population growth, annual food production should be doubled by 2050. This is not achievable by the current agronomical and breeding practices, due to impact of climate changes and associated abiotic stresses on agricultural production systems. The overall losses in food and fiber production due to abiotic stresses such as salinity, drought or flooding exceed US$170 billion p.a. and represents a major threat to global food security. To the large extent this is a result of past trends in breeding for higher yield on expense of tolerance, as the abiotic stress tolerance has been present in wild progenitors of modern crops but was lost during their domestication.
In this talk, I argue for a need for a major shift in our paradigm of crop breeding, focusing on the climate resilience, and call for a broader use of wild relatives as a major tool in this process. I also show that, while molecular tools are currently in place to harness a potential of climate-resilient genes present in wild relatives, a complex polygenic nature of tolerance traits remains a major bottleneck in this process. I show that the whole plant-based phenotyping will be not able to resolve this issue and argue for a need to shift towards cell-based phenotyping platforms allowing to assess in planta operation of key genes.
I then use several case studies to show how novel electrophysiological and imaging techniques can be used to overcome the above limitations and allow discovery of the candidate genes and/or QTLs conferring abiotic tolerance traits.

KEYWORDS: salinity, drought, waterlogging, ion homeostasis, membrane transporters

NEEDLES IN THE STRESSED HAY: CAN WILD BARLEY ALLELES CONTRIBUTE TO YIELD ABIOTIC TOLERANCE?

Eyal Fridman
Agricultural Research Organization (ARO), the Volcani Center, Bet Dagan, Israel
fridmane@agri.gov.il

Co-Author:

Ayelet Kurtz-Sohn
Agricultural Research Organization (ARO), the Volcani Center, Bet Dagan, Israel; 2 R.H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel

The wild ancestor of current crops is almost automatically regarded as a rich un-tapped repertoire of alleles to confer better resilience to stress tolerance of the crop. This Pavlovian reaction is probably owing to the wild environmental origin, which in some cases is harsher than agricultural fields. Nevertheless, unlike biotic resistance, there are very few known cases where wild alleles contributed to abiotic stress tolerance for grain yield. The reason probably lies in the combination of over-use of transcriptomic data (of leaves), oversimplifying a complex source-sink phenotype such as grain yield, and difficulties linking controlled and field experiments.
In this talk, we will show how considering the source-sink relationship and utilizing allelic series within a new wild-interspecific cytonuclear multi-parental population (CMPP) hint to source robustness mechanisms underlying yield abiotic stress tolerance which are mediated by the wild HsDry2.2 locus. This allelic series also unravels potentially novel variation that overlooked in this locus. Furthermore, we will show and discuss the use of same CMPP for exploring plasmotype conditioning of nuclear wild alleles effects on circadian clock and yield traits, including zooming-in on chloroplast gene alleles possibly underlying barley stress tolerance.

KEYWORDS: Multi-Parent populations; QTL; Drought; Source-sink

THE TRANSCRIPTOME LANDSCAPE OF BARLEY UNDER DROUGHT STRESS REVEALS INSIGHTS INTO THE ROLE OF ALTERNATIVE SPLICING IN ADAPTATION TO STRESS

Agata Daszkowska-Golec
Institute of Biology, Biotechnology and Environmental Protection; Faculty of Natural Sciences, University of Silesia in Katowice, Katowice, Poland

agata.daszkowska@us.edu.pl

Co-Authors:

Anna Collin
Institute of Biology, Biotechnology and Environmental Protection; Faculty of Natural Sciences, University of Silesia in Katowice, Katowice, Poland

Wenbin Guo, Runxuan Zhang
Information and Computational Sciences, James Hutton Institute, Dundee DD2 5DA, Scotland, UK

Martin Mascher, Murukarthik Jayakodi
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Germany

Hannes Kollist, Egon Megas, Ebe Merilo
Institute of Technology, University of Tartu, Tartu, Estonia

The dynamics and complexity of the transcriptome regulate plant growth, development, and responses to external biotic and abiotic cues. The cellular transcriptome is comprised of transcripts derived from a combination of both transcriptional and post-transcriptional processes. Alternative splicing (AS) potentially increases the number of protein isoforms produced from multiexon genes and regulates gene expression through multiple mechanisms: altered translational efficiency of splice isoforms, nonsense-mediated decay, and miRNA-mediated mRNA degradation. It is also well-known that stress evokes alternative splicing events in plants to adjust their adaptation to unfavorable conditions.
Here we investigated the transcriptome of barley leaves in response to drought conditions and ABA treatment at seedling stage based on the Barley Reference Transcriptome BaRTv2.18. We found that more than half of expressed genes in barley showed drought or ABA-induced differential expression and/or alternative splicing. We identified core sets of genes regulated specifically by either AS or transcription upon drought stress and ABA treatment as well regulated by both treatment in similar manner. AS made a significant contribution to changes in the transcriptome with almost 20% of genes whose expression changes significantly undergoing AS under drought. The analysis of the RNA-seq drought response at the transcript level identified more than 1000 protein-coding genes which were only regulated by AS with no significant changes in expression at the gene level. Currently, detailed analysis is performed to understand better the alternative splicing as a part of barley drought response and adaptation.

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KEYWORDS: barley, transcriptome, alternative splicing, drought, abscisic acid

DROUGHT RESPONSE AND RECOVERY IN BARLEY: A ROLE FOR AUTOPHAGY AND ITS LINK TO RETROTRANSPOSON EXPRESSION DYNAMICS

Maitry Paul
Institute of Biotechnology and Viikki Biocentre, University of Helsinki, Finland
maitry.paul@helsinki.fi

Co-Authors:

Marko Jääskeläinen, Wei Chang, Jaakko Tanskanen, Alan Schulman
Natural Resources Institute (LUKE), Institute of Biotechnology and Viikki Biocentre, University of Helsinki, Finland

Ahan Dalal, Menachem Moshelion
The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, Hebrew University of Jerusalem, Israel

Optimization of the balance between carbon fixed and water lost is the essence of water use efficiency (WUE). In drought, the decline in water homeostasis generates a response by the plant at a critical soil water content (SWC) leading to stomatal closure, even in daylight, to reduce water loss. The associated dehydration and its consequences are commonly referred to as drought stress. Drought response involves multiple mechanisms and signalling cascades related to stress and the growth and development of plants. Studies in Arabidopsis indicate that induction of autophagy is needed for drought tolerance. Autophagy is an evolutionarily conserved mechanism for recycling damaged proteins and cellular organelles by transport to the vacuoles or lysosomes for degradation. Another process relatively less studied at the end of the drought is recovery or rewatering. Rewatering at the end of a drought may result in the resumption of normal diurnal stomatal opening, recovery of photosynthesis, and the resumption of growth.
The occurrence, degree, and rate of recovery strongly depend on the intensity and duration of drought and on the species. Drought response is also well known to induce transcription of transposable elements, particularly retrotransposons (RLXs). The transcription of RLXs is driven by the promoters and response elements in their long terminal repeats (LTRs). While stress induction of RLXs has been demonstrated, a combination of well-controlled and documented physiological responses connected to both genomic transcriptional analysis and quantitative RLX transcriptional data has not been earlier reported for drought and rewatering. Here, we have examined BARE1 transcription and translation in the context of genome-wide gene expression and physiological response on a lysimeter platform in four barley cultivars in order to understand the interaction of drought, rewatering and RLX activation. Gene expression has been subjected to network analysis to understand pathways to which BARE and other RLXs may be responding.

KEYWORDS: Drought, Recovery, BARE, Retrotransposon, Autophagy

ROOT-ZONE-SPECIFIC TRANSCRIPTOMIC REPROGRAMMING OF BARLEY ROOTS IN RESPONSE TO WATER DEFICIT

Alina Klaus
University of Bonn
aosthoff@uni-bonn.de

Co-Author:

Frank Hochholdinger
University of Bonn

Harsh environmental conditions and extreme weather events threaten crop productivity and lead to immense yield losses. In the past, drought events occurred more frequently than at the beginning of the 21st century. It is expected that due to global warming this trend will increase further which ultimately poses drought as the major menace to food safety in the near future. Understanding how crop plants react to such stresses and might even overcome them will help lay the foundation for improved food safety. In the present study, barley seedlings were treated with a solution containing PEG8000 (- 0,8 MPa) to simulate severe drought stress. Three different root tissues (root cap and meristem, elongation zone and differentiation zone) were harvested after 6, 24 and 48 hours and gene expression changes between treated and untreated plants were observed via RNA-Sequencing. The number of stress-responsive genes varied strongly between the different tissues and time points. After six and 48 hours, more than 5,000 and 3,000 genes were differentially expressed, respectively while after 24 hours only 900 DEGs (differentially expressed genes) were identified. Independent of time, the highest number of DEGs was found in the elongation zone, followed by the differentiation zone. Detected DEGs in the differentiation zone continuously showed a preference for downregulation, whereas DEGs in the other tissues were more frequently upregulated after 6 and 48 hours. A comparison of DEG sets between tissues for each time point detected almost no conserved genes, that were shared by all tissues. Instead, there were many DEGs unique to their respective tissue and only small overlaps between tissues.
GO enrichment analysis identified over 150 terms that were significantly enriched in at least one tissue-time combination. Among these, many are associated with oxidoreductase, hydrolase and transferase activities indicating numerous physiological adaptations in response to water deficit. Moreover, a weighted correlation network analysis detected several modules that showed positive trait relationships and thus, together with the information gathered during the DEG analyses, may provide further insight into adaptive mechanisms and pathways in distinct root tissues of barley seedlings subjected to water deficit.

KEYWORDS: barley, water deficit, root-zones, RNA-Seq

THE ANALYSIS OF BARLEY CBP20/CBP80 DOUBLE MUTANT EXPOSED TO DROUGHT STRESS AT THE SEEDLING STAGE

Agata Daszkowska-Golec
Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice

agata.daszkowska@us.edu.pl

Co-Authors:

Ewa Sybilska, Wiktoria Fus, Marek Marzec
Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice

Drought stress is one of the main factors limiting plants' growth, development, and yield. In the face of global warming, there is an urgent need to obtain new varieties of crops with a higher tolerance to water deficit. The CBC (Cap-Binding Complex) is a heterodimer composed of two subunits: small CBP20 (Cap-Binding Protein 20) and large CBP80 (Cap-Binding Protein 80). The CBC is involved in the conserved cell processes related to RNA metabolism and the response of plants to drought. Plant mutants in CBP20 and CBP80 display better adaptation to stress conditions. These mutants under drought show faster stomata closing and better photosynthesis efficiency than wild-type (WT).
Therefore, a research question was raised regarding the physiological and molecular response to the drought stress of the double mutant hvcbp20.ab/hvcbp80.b. For this purpose we developed double mutant and performed preliminary study of its response to drought stress at the seedling stage. The experiments were carried out using a unique hvcbp20.ab/hvcbp80.b double mutant together with single hvcbp20.ab and hvcbp80.b and its parent cv. 'Sebastian'. This experimental design allows us to compare the effect of a single mutation and the functional impairment of the entire CBC complex in barley. The physiological analysis of plants exposed to drought stress at the seedling stage included the measurement of chlorophyll fluorescence, the photosynthetic pigments content index, and the RWC (Relative Water Content) index was performed. It was also checked whether the expression of selected genes related to ABA metabolism and signaling altered in response to drought stress. Currently, the experiment to determine the response of mutants after rewatering is also in progress.
These results together indicate a better adaptation of the double mutant to the drought stress at the seedling stage when compared with WT. It's worth noting that the observed response of hvcbp20.ab/hvcbp80.b was like the single hvcbp20.ab and hvcbp80.b mutants. However, the results of the post-harvest analysis suggest that the double mutation in hvcbp20.ab/hvcbp80.b may affect its phenotype.
To sum up, the obtained results indicate the potential role of CBP20 and CBP80 genes in improving barley drought tolerance. The double mutation in the CBP20 and CBP80 is a particularly interesting point in research targeted to engineer more climate-change-resistant crops. In further study planned in our team we will investigate it in deeper context.

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KEYWORDS: drought stress, CBC, abscisic acid, Hordeum vulgare

THE ROLE OF ABA IN BARLEY RESPONSE TO DROUGHT AT THE PRE-FLOWERING STAGE

Agata Daszkowska-Golec
Institute of Biology, Biotechnology and Environmental Protection, University of Silesia in Katowice, Katowice, Poland

agata.daszkowska@us.edu.pl

Co-Authors:

Anna Collin, Hubert Matkowski, Ewa Sybilska, Oliwia Krol, Asmarany Biantari, Agata Daszkowska-Golec
Institute of Biology, Biotechnology and Environmental Protection, University of Silesia in Katowice, Katowice, Poland

Drought stress is one of the most serious threats for global production of cereals. Barley is widely cultivated cereal exhibiting high level of drought tolerance. Therefore, it can serve as the model for other cereals for studying mechanisms of adaptation to stress. Abscisic acid (ABA) is known as the crucial phytohormone regulating plant response to drought at physiological and molecular levels. In our studies, we pre-treated barley plants with ABA and then applied drought stress at pre-flowering stage to check if ABA stimulates barley drought response.
We studied plants (1) growing under optimal water conditions, (2) treated with ABA, (3) pre-treated with ABA and then treated with drought, and (4) treated with drought. Physiological and molecular analysis allowed us to elucidate different types of molecular responses depending on growing regime. We observed better photosynthesis performance in ABA pre-treated plants under drought stress, when compared to those plants not pre-treated with ABA before drought occurred. Physiological and transcriptomic analyses allowed us to distinguish between treatment variants. Most profoundly observed traits were related to photosynthesis. Currently, we try to find out if observed differences are related to modification of alternative splicing. Our results shed a new light on ABA function in plant drought response and demand further studies.

Aknownledgements: This work was supported by the National Science Centre, Poland project SONATA BIS10 '(QUEST) Quest for climate-smart barley - the multilayered genomic study of CBC function in ABA signaling' (2020/38/E/NZ9/00346).

KEYWORDS: barley, ABA, drought, pre-flowering stage

WORLDWIDE ANALYSIS OF TEMPERATURE AND DAY LENGTH CUES AFFECTING FLOWERING IN BARLEY

Shakhira Zakhrabekova
Department of Biology, Lund University, Sölvegatan 35B, 22362 Lund, Sweden
shakhira.zakhrabekova@biol.lu.se

Co-Authors:

Silva W.
Centre for Environmental and Climate Research, Lund University, Sölvegatan 37, 22362 Lund, Sweden

Johansson J., Hansson M.
Department of Biology, Lund University, Sölvegatan 35B, 22362 Lund, Sweden

Lundquist U.
The Nordic Genetic Resource Center, Smedjevägen 3, 230 53, Alnarp, Sweden

Shoeva O.
Institute of Cytology and Genetics, Prospekt Akademika Lavrent'yeva, 10, Novosibirsk, Russia

Göransson M.
Agricultural University of Iceland, Hvanneyrabraut, 311 Hvanneyri, Iceland

Due Jensen J.
Nordic Seed A/S, Grindsnabevej 25, 8300 Odder, Denmark

Tondelli A.
Council for Agricultural Research and Economics (CREA), Research Centre for Genomics and Bioinformatics, via San Protaso 302, 29017 Fiorenzuola d'Arda, Italy

About 1100 different early barley mutants have been isolated by Swedish researchers and the mutants were designated as praematurum with the symbol mat. For the drastic mat mutants, allelism tests were conducted which distributed early mutants to 9 different loci. Mutants affected in mat-a locus has a special property which were denoted as "photoperiod insensitive".
In 2013 we decided to test four different mat mutants: mat-a.8, mat-b.7, mat-c.19, mat-e.18 and their mother cultivar Bonus in Russia since growing period in many regions of Russia is shorter and require early maturity plants. For a long time mat-a.8 was considered as the earliest mutant, but during our experiment in Russia we realized that the relative earliness between the mutants depends on when the mutants are planted and where they are growing. When plants were sown in Russia, one month later than usual we found that the mat-c.19 mutant was the first to flower.
This interesting discovery inspired us to perform a worldwide mat mutant experiment and collect phenology data of the four mutants and their mother cultivar at different locations and plant them at different time points. This will reveal how the different genes contribute to fitness of plants in a changing climate. Different locations used so far include Italy, Island, Russia, South Sweden, Denmark and Finland.

KEYWORDS: praematurum, ELF3, centroradialis, earliness, TFL1

GENOME WIDE ASSOCIATION STUDIES FOR YIELD COMPONENTS, PHYSIOLOGICAL, AND GRAIN MALTING TRAITS UNDER LATE HEAT CONDITIONS IN NORTHERN PLAINS OF INDIA ON GLOBAL BARLEY (HORDEUM VULGARE L.) COLLECTION FROM ICARDA

Ramesh Pal Singh Verma
Indian Institute of Wheat and Barley Research, Karnal India
rpsvdwr@hotmail.com

Co-Authors:

Swati Verma, Shikha Yashveer, Yogender Kumar
CCSHAU, Hisar, India

Sajid Rehman, Sanjaya Gyawali
ICARDA Morocco

Shiaoman Chao4
ARSUSDA, Fargo, ND, USA

Ashutosh Sarker
ICARDA India

Barley (Hordeum vulgare L.) is an important cereal crop grown in many regions of the world since ancient times. Heat stress in particular at the post-heading period, causes significant decrease in grain yield and quality especially in the malt barley crop in northern plains of India. Understanding molecular genetic basis for changes in physiological processes affecting the quantity and quality of grain under late heat stress for finding genetic diversity across genotypes is required to develop novel cultivars with tolerance to heat stress. A global panel of 316 different barley genotypes (AM2017), received from ICARDA was evaluated in two consecutive seasons, 2017-18 and 2018-19, at CCSHAU, Hisar, under timely (normal) and late sown (heat stress) conditions. Seven agro-morphological, four physiological and five grain malting quality traits were investigated. The 50 K iSelect Illumina Barley SNP array genotypic data of the AM2017 panel were used to estimate marker-trait associations (MTAs). The phenotypic data revealed a significant decrease in trait performance under the LS condition, while several genotypes were observed as tolerant with minimum heat susceptibility indices. A total of 307 Putative QTL were identified as being linked with these traits analyzed based on the estimated MTAs and linkage disequilibrium (LD) degradation seen in the genome. In all 21 most robust QTL for traits like heading days (5), maturity days (3), plant height (2), spike length (1), spikelets/spike (1), 1000gw (3), tillers/m (1), relative water content (1), hectolitre weight (1), % protein (1), and starch (2) were found under both normal and heat stressed conditions. Several new QTL were also found which are not yet reported. The combination of SNP studies and changes in physiological characteristics generated valuable information on genetic areas involved in heat stress tolerance.

KEYWORDS: Barley, heat tolerance, molecular markers, malting quality, physiological traits

THE MOLECULAR MECHANISM OF TEMPERATURE-RESPONSIVE INFLORESCENCE DEVELOPMENT IN BARLEY (HORDEUM VULGARE)

Tianyu Lan
Heinrich Heine Univerisity, Düsseldorf, Germany
tianyu.lan@hhu.de

Co-Author:

Maria von Korff.
Heinrich Heine Univerisity, Düsseldorf, Germany; CEPLAS-Cluster of Excellence on Plant Sciences

Improving the yield stability of cereal crops with a view to global climate change is a central issue in the 21st century. High ambient temperature is one of the leading abiotic stresses that limits the productivity of temperate cereals, in particular wheat and barley. It has been shown that high ambient temperatures can impair inflorescence development and floret fertility, and thus are a major cause of yield loss in barley. We have identified a major photoperiod responsive gene, Ppd-H1, known to interact with high ambient temperature to control inflorescence development and grain number per spike in barley.
However, the mechanisms that underpin the temperature-responsive inflorescence development are not fully understood. In this study, we have established that Ppd-H1 interacts with high ambient temperature to control the activity of the inflorescence meristem and thus the rate and duration of spikelet and floret primordium initiation. To unravel the molecular control of the inflorescence meristem under high ambient temperature, we conducted transcriptome profiling on developing inflorescences and leaves of genotypes differing at Ppd-H1 under different ambient temperatures.
We have identified floral homeotic and hormone responsive genes that were mis-regulated by high temperature in a Ppd-H1 dependent manner. Further analysis on auxin reporter lines has shown that hormone homeostasis in the floral organs was affected by the interaction of Ppd-H1 with high ambient temperature. These datasets and findings provide a valuable resource for future investigations into the complex regulation of inflorescence development in barley under high ambient temperatures.

KEYWORDS: high ambient temperature, inflorescence development, transcriptome profiling

EFFECT OF HIGH AMBIENT TEMPERATURE ON PLANT GROWTH AND REPRODUCTIVE DEVELOPMENT IN BARLEY CULTIVARS

Kumsal Ecem Çolpan
Institute of Plant Genetics, Heinrich-Heine-University Düsseldorf
kumsal.colpan@hhu.de

The increase in the average ambient temperature threatens crop production worldwide. As one of the most important cereal crops, barley (Hordeum vulgare) is an important target to generate cultivars that are tolerant to high ambient temperatures. Recently, a natural mutation in PPD-H1 prevalent in spring barley (ppd-H1) has been reported to cause delayed flowering and impaired reproductive growth under high ambient temperature, while the introgression lines carrying the wild type Ppd-H1 prevalent in winter barley show accelerated flowering and reproductive growth. Furthermore, the spring barley genotype (ppd-H1) mutants showed reduced numbers of grains and florets while the fertility and grain set were not significantly affected by high ambient temperature in introgression lines.
These findings suggest that PPD-H1 is a promising target for the generation of barley cultivars with improved grain set under high ambient temperature. However, the underlying molecular mechanisms behind Ppd-H1 mediated regulation of flowering time as well as reproductive development under high ambient temperatures remain unclear. Ppd-H1 is controlled by phytochromes. A natural variation on HvPYHTOCHROME C has been reported to interact with Ppd-H1 to accelerate flowering under different photoperiods. However, little is known about their interaction with high ambient temperatures.
Therefore, I study the molecular and genetic components involved in the regulation of reproductive development in barley. I analyse the genetic, hormone and metabolite networks in shoot apical meristem (SAM) which control the spike development downstream of PPD-H1 and PHYC under different ambient temperatures.

KEYWORDS: high temperature, reproductive development, PPD-H1, phytochrome, fertility

RESPONSES OF BARLEY TO HIGH AMBIENT TEMPERATURE ARE MODULATED BY VERNALIZATION

Ana M. Casas
EEAD-CSIC
acasas@eead.csic.es

Co-Authors:

Helga Ochagavía, Ernesto Igartua
EEAD-CSIC

Tibor Kiss, Ildikó Karsai
Agricultural Institute, Centre for Agricultural Research, ELKH, Martonvasar, Hungary

Ambient temperatures are increasing due to climate change, affecting crop development and production. Flowering time is a key factor for adaptation of barley (Hordeum vulgare L.) and, therefore, developmental its responses to rising temperatures must be explored.
This study was carried out with eight near isogenic lines of barley, differing at the VRN-H1, VRN-H2 and PPD-H1 genes, representing different growth habits, kindly contributed by Dr. Ben Trevaskis (CSIRO). The lines were exposed to treatments with and without vernalization, and then grown at two temperature regimes (18° and 25°C), in long days (16h). Days to Z31 (first node detectable, starting of stem elongation) and Z49 (awn appearance) were recorded. Inflorescence traits related to yield were measured at maturity. Unvernalized plants carrying winter vrn-H1 and VRN-H2 alleles did not reach jointing. Lines with recessive ppd-H1 displayed delayed development compared to lines with the sensitive PPD-H1 allele, across the two growth phases considered. High temperature delayed flowering in all unvernalized plants. For vernalized plants, however, the response to high temperature was dependent on the PPD-H1 allele they carried.
This finding evidenced an interaction between PPD-H1, temperature and vernalization. At the high temperature, PPD-H1 lines in spring backgrounds (VRN-H1-7) yielded more, whereas lines with recessive ppd-H1 were best in vrn-H1 background. This study revealed new information that will support breeding high-yielding cultivars with specific combinations of major adaptation genes suitable to future climatic conditions.

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KEYWORDS: barley, high temperature, vernalization, PPD-H1

ENHANCING WINTER SURVIVAL IN AUTUMN-SOWN BARLEY

Brian Steffenson
Department of Plant Pathology, University of Minnesota
bsteffen@umn.edu

Co-Authors:

Ahmad Sallam
Department of Plant Pathology, University of Minnesota

Kevin Smith
Department of Agronomy and Plant Genetics, University of Minnesota

Climate changes leading to higher summer temperatures can adversely affect cool season crops like spring barley. In the Upper Midwest region of the United States, one option for escaping this stress is to plant winter or facultative cultivars in autumn and harvest in early summer before the onset of high temperature stress. However, the major challenge in breeding such cultivars is incorporating sufficient winter hardiness to survive the extremely low temperatures that commonly occur in this production region.
The recent discovery of extraordinary winter hardiness in barley germplasm from the N. I. Vavilov genebank offers great hope for breeding for enhanced levels of this critical trait. The two barley panels VIR-LTT (Sallam et al., 2021) and CAP-LTT (Muñoz-Amatriaín et al., 2020) were genotyped with the Illumina 9K single-nucleotide polymorphism (SNP) chip and evaluated for winter survival (WS) in multiple locations. Genome-wide association mapping was performed independently by two different research groups to identify several novel quantitative trait loci (QTL). Twelve significant associations for WS were identified in the VIR-LTT panel, including the previously reported frost resistance gene FR-H2. The CAP-LTT panel was used previously to identify fifteen loci associated with WS including the FR-H1, FR-H2, and FR-H3 genes. Multi-allelic haplotype analysis implemented in the haplo.stats package was used to determine haplotype parameters and associations with WS (Sallam et al., 2021). Multi-allelic haplotype analysis in both panels revealed the most favorable alleles for all the previously identified loci and individuals carrying these alleles (Sallam et al., 2021). To accumulate favorable winter hardiness alleles into barley breeding lines, we initiated the development of a Multi-parent Advanced Generation Inter-Cross (MAGIC) population using diverse parents from the VIR-LTT and CAP-LTT panels. Using the average WS performance of accessions in both the VIR-LTT and CAP-LTT panels along with complementary favorable haplotype alleles, eight founder parents with excellent WS and maximum number of favorable haplotype alleles were identified. The founder parents were intercrossed in a two-way crossing scheme followed by the four-way and eight-way crosses steps. The population will be evaluated for winter survival and agronomic traits in several environments in the United States. This population design will facilitate the precise positioning of winter hardiness genes and generate offspring carry favorable alleles at all known WS QTL.

KEYWORDS: Barley, Winter Survival, MAGIC

WATER USE EFFICIENCY OF A SPRING 2-ROW BARLEY POPULATION ASSESSED IN THE PLANTARRAY PHYSIOLOGICAL PHENOTYPING PLATFORM

Alessandro Tondelli
CREA, Research centre for Genomics and Bioinformatics, Fiorenzuola d'Arda, Italy
alessandro.tondelli@crea.gov.it

Co-Authors:

Ahan Dalal, Menachem Moshelion
The R.H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel

Giulia Alussi, Luigi Cattivelli
CREA, Research centre for Genomics and Bioinformatics, Fiorenzuola d'Arda, Italy

Drought is the most significant environmental stress in agriculture and its occurrence and severity are expected to increase due to climate changes. In the Mediterranean region, a change in the rainfall regime, especially a long period of drought between two rainfall events, will have a profound impact on cereal productivity, which are strategically and economically important crops.
Consequently, improving cereal yield under drought is a major goal of plant breeding. One of the major challenges in the process is overcoming the genotype-phenotype gap. In the frame of the FACCE-ClimBar project, a set of European spring barley cultivars were screened for their water use efficiency in the PlantArray physiological phenotyping platform. The two-rowed cultivars Chanell (released in Denmark in 2006) and Formula (Sweden, 1987) displayed isohydric/high WUE and anisohydric/mid WUE behaviours, respectively. Noticeably, they harbour the same alleles at major flowering genes, hence phenology does not impact on stress response. Here, 150 Doubled Haploid (DH) lines from a Chanell x Formula Doubled Haploid population was genotyped with an Infinium Illumina 15K SNP array and preliminarily tested in the same PlantArray platform, in order to detect QTLs controlling the differential response to water limitation.

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KEYWORDS: Drought, QTL, PlantArray

INVESTIGATION OF ALUMINUM TOXICITY RESPONSE OF LITHUANIAN BARLEY CULTIVARS

Vilius Mensonas
Institute of Biosciences, Life Sciences Centre, Vilnius University, Saulėtekis Ave. 7, 10257, Vilnius, Lithuania
vilius.mensonas@gmc.vu.lt

Co-Authors:

Raimondas Šiukšta
Institute of Biosciences, Life Sciences Centre, Vilnius University, Saulėtekis Ave. 7, 10257, Vilnius, Lithuania

Algė Leistrumaitė
Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, Institutas ave. 1, Akademija, 58344, Kėdainiai, Lithuania

Rapidly changing climate and other anthropogenic or natural causes often render swathes of previously utilized farmland unusable. Together with the deterioration of already used land, having to wrestle with unfavorable growing conditions makes the expansion of farmland difficult, particularly in developing countries, accentuating the problem of food shortages as well as limiting the agricultural industry. One of the major problems that farmers face due to changing climate or pollution is soil acidity. The perhaps lesser-known side of the same soil acidity coin is aluminum poisoning. In fact, it is aluminum poisoning which is the main reason for plant stress in acidic environments, rather than low pH itself. In acidic soil, aluminum ions become soluble and cause a variety of disturbances to plant homeostasis, from disrupting ion transport to interfering with the cell cycle.
In this study, we analyzed barley, in order to find cultivars, which are naturally resistant to aluminum poisoning. To achieve this, we subjected three days old Lithuanian and standard foreign barley seedlings to 2 mM and 8 mM concentrations of AlCl3 and measured biochemical and morphological parameters, such as shoot and root length, chlorophylls a, b and carotenoid concentrations, lipid peroxidation levels, aluminum levels in seedling roots, as well as root cell viability. Root and shoot length measurements showed a statistically significant ($p < 0.05$) reduction in length for both roots and shoots after 72 hours of aluminum stress, as well as visible deterioration of root quality with roots becoming rigid, torn and yellowish. Photosynthetic pigment assays, as well as lipid peroxidation analysis, did not produce any statistically significant differences between control and stress groups. Aluminum level in roots and root cell viability assays showed statistically significant differences between stress and control groups, with stress group roots containing more aluminum and unviable cells than their control counterparts. Using all morphometric and biochemical indicators we identified two most aluminum-resistant Lithuanian barley cultivars – ‘Ema’ and ‘Kirsna’, which in some experiments performed better than the standard ‘Bavaria’.
To complement biochemical and morphological analyses we also performed genetic analyses, targeting previously identified aluminum stress-related genetic markers in citrate transporter gene HvAACT1. Three markers, in particular, were analyzed, 5’ UTR insertion, 3’ UTR deletion and an SNP in position 1198, all associated with increased aluminum stress tolerance. None of our analyzed cultivars had the 5’ UTR insertion, and two – acidity resistant standard ‘Bavaria’ and Lithuanian ‘Noja’ – had both the 3’ UTR deletion and stress-resistant SNP variant at position 1198. The mismatch between the results of empirical and genetic analyses suggests that there are other, yet undiscovered, mechanisms of aluminum resistance in barley.

KEYWORDS: aluminum, soil acidity, barley seedling

THE HORDEUM GENUS: A RICH RESOURCE FOR ADAPTIVE GENETIC VARIATION

Michael Anokye
Institute of Plant Genetics, Heinrich-Heine-Universität Düsseldorf, 40225, Düsseldorf, Germany
Michael.Anokye@hhu.de

Co-Authors:

Maria von Korff Schmising
Institute of Plant Genetics, Heinrich-Heine-Universität Düsseldorf, 40225, Düsseldorf, Germany;

Philipp Westhoff
CEPLAS Metabolomics and Metabolism Laboratory (CMML), Heinrich-Heine-Universität Düsseldorf, 40225, Düsseldorf, Germany

Stanislav Kopriva
Institute for Plant Sciences, University of Cologne, D-50674, Cologne, Germany

The Poaceae family dominates many plant communities globally and as such are important for ecosystem stability and services. Due to their wide adaptability to different ecological zones including stress environments, they represent an interesting family to explore adaptation to diverse and changing environments. Here we focus on the diverse grass genus Hordeum originating from arid, stress prone, temperate environments of South America, and Eurasia as a source of valuable genetic diversity for improving crop plant performance under abiotic stresses. To test this hypothesis, we characterise phenotypic variation within and between Hordeum species together with cultivated barley (H. vulgare vulgare) to gain insight into the traits and trait complexes that enabled adaptation to different stress-prone environments.
Preliminary phenotyping of a large number of different shoot traits in garden experiment in Hordeum species could already show that variation in growth habit (annuals and perennials), shoot and leaf development existed within and between the species. While differences in growth habit and leaf traits has been connected to ecological strategies, “grow fast to escape stress versus grow slow to tolerate stress”, the extent to which growth habit and leaf traits relates to stress adaptation still require further investigation.
Combining field and greenhouse experiments, we will study natural genetic variation in shoot development (including growth rate, senescence pattern, stay green), leaf growth traits (leaf mass area, leaf nitrogen content, cell sizes, stomata density) and physiology (carbon assimilation, stomatal conductance, transpiration) of experimental populations of cultivated and wild Hordeum species. Together, we will also investigate shoot plasticity of the species via assessment of genotype by environment variations. In addition, differences in elemental composition, the “ionome”, of leaf and seed would be quantified. Overall, the goal is to unravel traits at the phenotypic level relevant for plant performance in stress-prone natural habitats characterized by complex and changing environmental conditions. The project will thus, contribute towards efforts in identification of traits beneficial for re-introduction from wild-relatives into modern breeding pools (rewilding). Here, we show preliminary results of leaf growth traits, shoot development and ionome quantification of leaves and seed samples during 2021 growing season in the field.

Aknownledgements: CEPLAS, iGradBio Graduate School

KEYWORDS: Shoot phenotyping, leaf growth dynamics, growth habit, drought tolerance, shoot plasticity

AGRONOMIC CHARACTERIZATION OF EUROPEAN HERITAGE COLLECTION (EXHIBIT)

Villő Bernád
School of Biology and Environmental Science, University College Dublin, Belfield, Dublin 4, Ireland
vill.bernad@ucdconnect.ie

Co-Authors:

Nadia Al-Tamimi, Patrick Langan, Tim Dempsey, Joey Henchy, Mortaza Khodaeiaminjan, Sónia Negrão
School of Biology and Environmental Science, University College Dublin, Belfield, Dublin 4, Ireland

Gary Gillespie, Mary Harty
Crop Sciences, School of Agriculture and Food Science, UCD, Belfield, Dublin 4, Ireland

Luke Ramsay, Kelly Houston, Joanne Russell, Robbie Waugh
The James Hutton Institute, Dundee, UK

Kevin P. Mcdonnel
Crop Sciences, School of Agriculture and Food Science, UCD, Belfield, Dublin 4, Ireland & Biosystems Engineering Ltd., NovaUCD, Belfield, Dublin 4, Ireland

Barley is the fourth most important cereal worldwide and is particularly important for food security. However, barley production is being hindered by increased environmental pressures such as waterlogging caused by climate change. Barley has the second largest germplasm collection in the world, yet this invaluable collection remains largely uncharacterized.
In this project we assembled the European Heritage Barley collection (ExHIBiT), which includes 348 lines of 2-row spring barley. The ExHIBiT collection comprises a mixture of landraces (~10%), formerly bred cultivars (~80%) and elite cultivars (~10%) and has been genotyped with 50K SNP array and cultivated at UCD Lyons Research Farm in 2020 and 2021. First year of field trials combined with genotypic data and population structure were used to establish a core-collection comprising 230 accessions.
During the second year of field trials the core-collection was assessed for several agronomic traits and screened for waterlogging tolerance. The Exhibit Core Collection was found to be agronomically diverse and include waterlogging tolerant accessions. Association analysis of agronomical traits is currently ongoing to validate the utility of the collection in future association mapping studies and further field trial is being carried out to confirm waterlogging tolerance and to identify candidate genes.

KEYWORDS: Abiotic Stress, Association mapping, Core collection, Waterlogging

BARLEY BREEDING AND NEW BREADING TECHNIQUES: A WAY FORWARD?

SITE-DIRECTED GENOME MODIFICATION IN BARLEY - METHODS AND APPLICATIONS

Jochen Kumlehn
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Germany
kumlehn@ipk-gatersleben.de

Site-directed genome modification triggered by RNA-guided Cas endonucleases offers unprecedented opportunities for the elucidation of gene functions and the improvement of crop performance. As an essential prerequisite for keeping pace with the rapid development of this technology, we have developed a modular and versatile vector system that is universally useful for mono- and dicot plants, and rests upon type IIS restriction enzymes, thereby allowing for complex cloning procedures in a comparatively straightforward way.
Taking advantage of this system, not only multiple guide RNAs can be simultaneously expressed but also newly emerging system components such as Cas derivatives with improved or novel functionality, e.g. Cas12a variants and engineered Cas9 enzymes with base editing capability or with relaxed specificity for the protospacer-adjacent motif, can be readily tested and utilized. In addition, polyethylene glycol-mediated transfection of protoplasts was shown to be a valuable means to put Cas endonuclease vectors to the test prior to their employment for targeted genetic modification at the plant level. It was further demonstrated that the multiple genetic modifications carried by the typically chimeric primary mutants can be perfectly separated and fixed in just one step by producing doubled haploid progeny.
We have been utilizing Cas endonuclease technology in barley to establish resistance to Bymoviruses and fungal pathogens, to modify plant height, spike and grain morphology as well as to improve qualitative features.

KEYWORDS: Base editing, CRISPR, Genome editing, haploid technology

GENOMIC-ASSISTED SPARSE MULTI-LOCATION TESTING TO INCREASE GENETIC GAIN IN BARLEY

Julian Garcia-Maldonado
Biodiversity and Crop Improvement Program (BICP), International Centre for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco
juligarmal@hotmail.com

Co-Authors:

Z. Kehel, K. el Shamaa, A. Visioni, M. Sanchez-Garcia
Biodiversity and Crop Improvement Program (BICP), International Centre for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco

S. Gezan
VSN International, Hemel Hempstead, United Kingdom

In recent years, breeders have been incorporating genomic predictions (GP) into their breeding programs as a useful, cost-effective, and versatile tool to increase genetic gain. Coupled with new experimental designs, genomic prediction maximizes the information quality and quantity per dollar invested. This is the case of genomic-assisted sparse multi-location testing, an experimental design that allows breeders to arrange multi-location trials having only a fraction of the genotypes present in all environments. In this design, the observed genotypes at each environment are used to produce genomic predictions of the non-planted ones and the overlapping genotypes across locations enable the genotype x environment (GxE) connectivity and its modeling. This results in an increase in selection accuracy and/or intensity and thereby higher genetic gains. This strategy has been adopted by the Global Barley Breeding Program of ICARDA as the standard approach for preliminary yield trials. To assess GP accuracies within and across locations, 1,000 new 2- and 6-row stage 1 entries of the Feed Barley for Arid and Semi-Arid Environments Mega-Product Line were assembled in a preliminary yield trial series with four locations. These lines were distributed in 340 plot sparse p-rep trials that included 212 unreplicated entries per location, 18 entries replicated within location (p-rep entries), 80 entries replicated across locations and 6 commercial checks replicated both within and across locations. These trials were planted in four diverse locations in Morocco and Lebanon, each identified as representative of a Target Population Environment. The phenotypic correlation among environments were all lower than r = 0.30. In order to maximize the kinship connectivity, the lines were distributed across locations based on the pedigree-based matrix of relationships. In addition, all 1,000 lines were genotyped using a marker diversity set of 96 SNP distributed across the genome and previously selected for their high minor allele frequency (MAF > 0.40) among the parents. Genomic predictions were calculated using GBLUP fitted using the ASReml-R, following a two-stages analysis. A scheme of 10-fold cross-validation was set up to calculate GP accuracies within environment, and the same approach was expanded to GP in multi-environment trials (MET). Different variables were added to the genomic models to improve the accuracy of the predictions. These included, population structure, pedigree information (hybrid matrix) and row-type. These were added both independently or combined into the models within environment and in the MET analysis. For within location cross-validation, the best results were obtained when only the hybrid matrix was included in the models. Final GxE genomic prediction accuracies ranged between 0.20 to almost 0.50.

Aknownledgements: The authors would like to thank the Arab Fund for Economic & Social Development (AFESD) for supporting this work from the project “Modernization of ICARDA breeding programs”

KEYWORDS: Genomic Prediction, sparse multilocation testing, genotype-by environment, genetic gain

NEW TOOLS FOR BARLEY GENOME EDITING

Wendy Harwood
John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK
wendy.harwood@jic.ac.uk

Co-Author:

Tom Lawrenson
John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK

Genome editing in barley to produce heritable, targeted mutations or 'knock-outs' was first reported by us in 2015. We used Agrobacterium-mediated delivery of editing components to immature barley embryos and our reported editing efficiencies at the time were 23%. We have now used CRISPR / Cas9 based editing techniques to produce knock-out mutations in over 100 barley target genes. Editing efficiencies have been boosted to very high levels through the application of novel nuclease variants.
We will describe the development and application of these nuclease variants as well as provide some data on the impact of construct architecture when editing multiple genes simultaneously. Gene targeting or 'knock-in' in crops is a valuable application of genome editing but it remains inefficient as it depends on the cell's homology directed repair pathway which is far less common than non-homologous end joining based repair, that typically leads to knock-outs.
We will consider how some of the improvements made to genome editing efficiencies might allow higher efficiencies of gene targeting in barley.

KEYWORDS: Genome editing, CRISPR/Cas, barley, novel nucleases

CURRENT STATUS AND FUTURE PROSPECTS FOR NBT REGULATION IN EUROPE

Alan Schulman
Natural Resources Institute Finland (LUKE) & Institute of Biotechnology, University of Helsinki
alan.schulman@helsinki.fi

In July 2018, the European Court of Justice (CJEU) ruled that genome-edited (GE) organisms are to be regulated as GMOs. This decision curtailed the development and application of editing tools such as CRISPR-Cas9 for practical breeding and novel food and feed across Europe. Nevertheless, GE methods are being exploited for improving varieties and foods, soon to be on the market, in the major trading partners, where it is not regulated as GM. Genetic variation induced through GE cannot in most cases be distinguished from either spontaneous mutations or those generated through traditional mutagenesis. Hence, GE products in international (and also internal EU) trade raises great difficulties for national authorities that regulate the import of foodstuffs, as no diagnostic tests specific to GE can be developed.
In response to such concerns, the European Commission (EC) has carried out an Impact Assessment ("Roadmap") through several phases during 2021—2022. This is expected to lead to a legislative initiative in the near future.
The presentation will summarize the policy and legal status and prospects for GE within the EU and the implications for barley researchers.

KEYWORDS: gene editing; NBTs; regulation; EU

TRAINING SET OPTIMIZATION FOR SPARSE PHENOTYPING IN GENOMIC SELECTION

Julio Isidro Sánchez
Technical University of Madrid, Madrid, Spain
j.isidro@upm.es

Co-Author:

Deniz Akdemir
CIBMTR (Center for International Blood and Marrow Transplant Research), National Marrow Donor Program/Be The Match, Minneapolis, MN, USA

Genomic Selection (GS) is considered the most promising tool for genetic improvement ofthe complex traits controlled by many genes, each with minor effects because (i) GS can increase the rates of genetic gain through increased accuracy of estimated breeding values, (ii) significantly shorter breeding cycles, and (iii) the better utilization of available genetic resources through genome-guided mate selection.
Breeders test candidate genotypes in multi-year and multi-location trials to select superior genotypes with high performance. This approach limits the number of variety candidates to be tested, and it is the main cause of the fact that plant breeding programs are time and cost-intensive. A breeding tool that combines the power of GS and the potential of an extensive collection of germplasm, assisted by new technologies, will offer promise in crop breeding to contribute to global food security because it can accelerate the generation interval by reducing the generation time in plant breeding programs. The design of the training set (TRS) in GS is one of the key steps in the implementation of GS in plant and animal breeding programs mainly because (i) TRS optimization is critical for the efficiency and effectiveness of GS, (ii) breeders test genotypes in multi-year and multi-location trials to select the best- performing ones.
In this framework, TRS optimization can help to decrease the number of genotypes to be tested and, therefore, reduce phenotyping cost and time, and (iii) we can obtain better prediction accuracies from optimally selected TRS than an arbitrary TRS. Here, we review the lessons learned from training population optimization in plants and the major challenges associated with the optimization of GS including population size, the relationship between training and test set (TS), update of TRS, and the use of different packages and algorithms for TRS implementation in GS. Finally, we describe general guidelines to improving the rate of genetic improvement by maximizing the use of the TRS optimization in the GS framework.

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KEYWORDS: training set optimization, genomic selection, genome-wide markers, statistical design, sparse phenotyping, genomic prediction, mixed models

GENETIC DETERMINANTS OF MALTING QUALITY TRAITS IN A BARLEY POPULATION REPRESENTATIVE OF ELITE BREEDING IN SOUTH AMERICA

Lorena Cammarota-Ricco
Programa de desarrollo de las ciencias básicas, biologia, genética. Universidad de la República
lorena.cammarota@gmail.com

Co-Authors:

Silvina Baraibar
Instituto Nacional de Investigación Agropuecuaria, Estación Experimental La Estanzuela, Ruta 50, Km11, Colonia, Uruguay

Fernanda Cardozo
Maltería Uruguay S.A. Ruta 55, Km26, Ombúes de Lavalle, Uruguay

Blanca Gómez-Guerrero
Latitud, Fundación LATU, Av Italia 6201, Montevideo 11500, Uruguay

Lucía Gutiérrez
Department of Agronomy, University of Wisconsin-Madison, 1575 Linden Dr., WI, 53706, USA

Valeria Lanaro
Laboratorio Tecnológico del Uruguay- LATU, Av Italia 6201, Montevideo 11500, Uruguay

Andres Locatelli
CENUR Litoral Norte, Universidad de la República, Ruta 3, Km363, Paysandú 60000, Uruguay

Gastón Quero
Departamento de Biología Vegetal, Facultad de Agronomía, Universidad de la República, Garzón 809, Montevideo, Uruguay

Fernanda Pardo, Mercedes Sayas
Maltería Oriental S.A., Camino Abrevadero 5525, Montevideo 12400, Uruguay

Maximiliano Verocai, Ariel J. Castro
Departmento de Producción Vegetal, Facultad de Agronomía, Universidad de la República, Ruta 3, Km363, Paysandú 60000, Uruguay

Barley is the second spring crop in Uruguay, with the export of malting barley as its main destiny. High malting quality is a key target for local and regional breeding programs and the industry requirements have increased in recent years, becoming a great challenge for the maltinghouses to meet the expectations of importers malt quality specifications with malt from local cultivars. Barley production in the region has a strong dependence on modern European germplasm that combines high grain yield potential and excellent malting quality. Local breeding programs try to combine this quality and yield potential with the adaptation of elite local materials. Knowledge about the genetic components of major malting quality traits is very useful for efficient barley breeding but little information about malting quality QTL in germplasm representative of local breeding programs is available. The goal of our study was to identify chromosome regions and candidate genes associated with four key quality traits (malt extract, soluble nitrogen, beta-glucans, and grain protein) in samples obtained in three environments from germplasm representative of the crosses used in local breeding programs. To achieve that we used a population of 145 double haploid lines obtained from crosses between modern European cultivars and local well-adapted germplasm.

The population was genotyped by the Illumina barley 50K iSelect SNP array resulting in 6220 informative SNPs covering all chromosomes. We performed a GWAS analysis using a mixed model considering the population (Q) through PCA analysis (selected as the most appropriate for our study). Our preliminary results indicate a total of 248 marker/trait associations defining (considering coincidence) a total of 24 QTLs. 13 of them were located in two hotspot zones in chromosomes 2H and 5H. Several QTLs were detected in more than one environment and were associated with more than one trait. Both European and local parentals contributed favorable alleles, opening the chance to achieve transgressive segregants for high quality. In the cases where the same region affects more than one trait we did not detect favorable alleles in repulsion phase, a fact which also facilitates breeding efforts. The same population was studied in a parallel project for agronomic traits and we did not detect coincidence between QTLs affecting both types of traits, allowing the improvement of malting quality without affecting agronomic adaptation. Overall QTL location was limited when compared with other studies, which can be related to the fact that the crosses were elite x elite and we expected that some major QTLs must be fixed on them. Our results provide useful information as the population used allowed a more direct utilization of the results in breeding. They highlight, also, the potential limitations of QTL detection for further improvement of complex traits on elite germplasm.

KEYWORDS: GWAS, malting quality, QTL

COMPARATIVE STUDY OF POWDERY MILDEW RESISTANCE BREEDING STRATEGIES IN BARLEY

Liina Jakobson
Department of Plant Biotechnology, Estonian Crop Research Institute, Jõgeva, Estonia
liina.jakobson@etki.ee

Co-Authors:

Kai Ilves, Ljudmilla Timofejeva, Kristiina Laanemets
Department of Plant Biotechnology, Estonian Crop Research Institute, Jõgeva, Estonia

Ülle Tamm
Department of Jõgeva Plant Breeding, Estonian Crop Research Institute, Jõgeva, Estonia

Bulat Islamov
Department of Plant Protection, Estonian Crop Research Institute, Jõgeva, Estonia

Powdery mildew outbreaks often result in severe harvest loss due to reduced grain yield and quality in barley (Hordeum vulgare). Natural as well as chemically induced mutant lines carrying recessive mlo (mildew resistance locus o) alleles display resistance to the foliar powdery mildew disease-causing fungi. Marker-assisted selection for mlo has been successfully employed in agriculture over decades. Here we show a comparative study of powdery mildew resistance breeding strategies in barley. We implement marker-assisted breeding in parallel with CRISPR-Cas9-mediated precision breeding. Based on the facilities available at Estonian Crop Research Institute, we aim to compare feasibility, time cost and adaptability of these tools in barley breeding.

For marker-assisted breeding strategy, we have chosen two donor varieties ('Selene', 'Spectra') with the natural allele mlo-11 originating from Ethiopian landrace and a donor variety ('Alexis') with EMS mutagenesis-derived allele mlo-9. As the acceptor we used Estonian varieties 'Maali' and 'Tuuli' known to be susceptible to powdery mildew. Selection of plants with the desired alleles has been verified after each of the four back-crossings by PCR-based marker analysis. F4 plants obtained from five different crosses were self-pollinated and altogether 1824 F5 plants were genotyped. From those F5 plants 354 (19%) were subjected to further phenotyping, all homozygous for the respective mlo allele. Initial inoculation tests with four local isolates of Blumeria graminis f. sp. hordei (Bgh) let us conclude that all tested 25 barley lines from F5 were more resistant than the control genotype 'Jyvä'. Large-scale field-testing of promising breeding lines and subsequent inoculation tests are planned for the growth season 2022.

For the precision breeding strategy, we have induced mlo loss-of-function alleles with CRISPR-Cas9. At first, we applied Agrobacterium-mediated transformation on variety 'Golden Promise'. We have created three vectors, each with two different gRNA sequences targeting MLO (MLO-T1T2, MLO-T3T4, MLO-T1T4). Altogether 200 embryos have been transformed and seven viable T0 plants were recovered. We plan to apply the optimized precision breeding method also on the Estonian elite varieties 'Maali' and 'Tuuli'.

In conclusion, the need to cut down on pesticide use and to increase the yield and crop quality is strongly affecting plant biotechnology and breeding in cereals. Here we show our first results from a comparative study between marker-assisted breeding and precision breeding in barley aiming to create new varieties resistant to powdery mildew, which would need less pesticides and have higher yield.

KEYWORDS: barley, powdery mildew resistance, MLO, precision breeding, marker-assisted selection

OPTIMIZING BARLEY GENOME EDITING WITH INSIGHTS FROM DDPCR

Claus Krogh Madsen
Crop Genetics and Biotechnology, Department of Agroecology, Aarhus University, Forsøgsvej 1, DK-4200 Slagelse

ClausKrogh.Madsen@agro.au.dk

Co-Authors:

Tobias Hanak, Jovana Janjic, Henrik Brinch-Pedersen
Crop Genetics and Biotechnology, Department of Agroecology, Aarhus University, Forsøgsvej 1, DK-4200 Slagelse

Digital droplet PCR (ddPCR) is based on the partition of the sample into thousands of nano-liter droplets before PCR. Most droplets will contain 0-2 template molecules that will be amplified in a discrete reaction. This allow absolute quantification of DNA templates by the application of Poisson statistics to the positive droplet count. The use of differentially labeled probes make it possible to determine whether or not two marker templates are present on the same molecule. These properties are utilized in the so called drop off assay for mutation detection. One dual labeled probe is designed on target, e.g. of a CRISPR-Cas9 construct. A second (reference) dual labeled probe with a different chromophore is designed to anneal nearby. Finally, primers are designed to flank both probes. In this assay, a wild type template generates signal from both probes whereas a mutated template only returns one signal from the reference probe. The partition of the sample, which is key to ddPCR, enable the detection of mutated molecules in a large excess of wild type template. We have found that it is possible to detect mutations in barley callus DNA already one week after transformation. This provides an early indication of the efficiency of new protospacer designs. It is also possible to test and optimize different vector designs, Cas variants or Agrobacterium strains. We are currently using this tool to improve genome editing of H. vulgare and H. spontaneum. Another useful application is determination of gene copy number variation. This is done by comparing the number of positive droplets from a probe targeting known single copy gene to a probe targeting the gene of interest. Applied to transgenics, this allow accurate and fast determination of T-DNA copy number. This information is useful to avoid false positives and to explain and predict segregation patterns.

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KEYWORDS: Digital Droplet PCR, CRISPR-Cas, genotyping, genome editing

PROMOTER BASHING AND MODIFICATION OF CRES IN THE HVNEP-1 PROMOTER FOR IMPROVED EXPRESSION AND FHB DISEASE RESISTANCE

Zelalem Eshetu Bekalu
Aarhus University, Department of Agroecology, Slagelse DK-4200, Denmark
Zelaleme.bekalu@agro.au.dk

Co-Authors:

Inger Holme, Henrik Brinch-Pedersen
Aarhus University, Department of Agroecology, Slagelse DK-4200, Denmark

Fusarium head blight (FHB) is an important disease of the major small grain cereals, mainly wheat and barley. Recently, the barley nepenthesin-1, HvNEP-1 gene has been identified as a resistance gene against FHB. Overexpressionssion of HvNEP-1 in the endosperm of barley grains substantially reduced FHB severity and disease progression. It also significantly reduced the accumulation of important mycotoxins including DON, NIV, and ZER in the grains of transgenic lines compared to WT controls.
Therefore, to include HvNEP-1 in the resistance-breeding program, the availability of natural variation in the HvNEP-1 gene expression should be investigated. Promoter bashing, modulation of Cis-regulatory elements (CREs), and modification of binding sites for the repressors have been the targets for improved gene expression. Here, we employ CRISPR Cas9 mediated genome editing for the modification of promoter CREs and Kozak consensus motifs, and 3'UTR resident CREs that are involved in the processing of mRNA and translation.

KEYWORDS: FHB, HvNEP-1, resistance breeding, CRISPR Cas9, promoter bashing, CREs

GEARED - GENOME EDITING-ACCELERATED RE-DOMESTICATION OF WILD BARLEY HORDEUM VULGARE SSP. SPONTANEUM AS A TOOLKIT FOR CURRENT AND FUTURE CHALLENGES.

Tobias Hanak
Aarhus University, Department of Agroecology
tobias.hanak@agro.au.dk

Co-Authors:

Claus Krogh Madsen, Jovana Janjic, Henrik Brinch-Pedersen
Aarhus University, Department of Agroecology

Barley is a major crop in Europe and an important resource for animal feed and beer production. In order to arm this resource to cope with the upcoming climate changes, new resilient cultivars are needed.
By new breeding techniques, it is feasible to alter major domestication genes for traits like brittleness, spike-architecture, flag-leaf size or caryopsis in wild barley (Hordeum vulgare ssp. spontaneum) and thereby create new germplasm for breeding. Currently, wild barleys from drought areas in Israel are being characterizing with respect their resilience towards abiotic and biotic stress. Preliminary results demonstrates significant post-drought regeneration capabilities and resistance to nematodes in wild barley. In order to facilitate genome editing in wild barley, an Agrobacterium mediated transformation system is established.
So far, a protocol for Agrobacterium-mediated transformation of H. vulgare ssp. Spontaneum, enabling to generate a moderate amount of regenerated plants was established. Gene editing events needs to be evaluated.

KEYWORDS: NBT, Re-domestication, transformation, wild barley

THE PLAMOTYPE AS A SOURCE FOR PHENOTYPIC DIVERSITY UNDER STRESS IN BARLEY

Eyal Bdolach
Agricultural Research Organization (ARO), the Volcani Center, Bet Dagan, Israel; Department of Life Sciences, Ben-Gurion University, 84105, Beer-Sheva, Israel

eyalbd@post.bgu.ac.il

Co-Authors:

Schewach Bodenheimer
Agricultural Research Organization (ARO), the Volcani Center, Bet Dagan, Israel; R.H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel

Eyal Fridman
Agricultural Research Organization (ARO), the Volcani Center, Bet Dagan, Israel

Robustness in face of environmental changes is considered a key feature of circadian clock systems yet its genetic basis is poorly understood. One possible source for genetic diversity in barley is the wild ancestor Hordeum vulgare sps. spontaneum. This study investigates the diversity of plasmotypes (chloroplast and mitochondria) in the wild as a source for phenotypic diversity, including that of the circadian clock outputs, under stress. To test possible causal effects of plasmotype diversity we generated the Cytonuclear Multi-Parent Population (CMPP). This homozygous resource includes segregation of both nucleotype and plasmotype of ten wild barley in a common cultivated background. Furthermore, during the development of the CMPP, several BC1 lines were self-pollinated four times (BC1S4) and tested both on the SensyPAM, circadian clock phenomics platform, and in the nethouse. We scored phenotypes under ambient temperature and high temperature for fitness traits, as well for circadian clock rhythmicity under optimal (22ºC) and high (32ºC) temperatures. We generated and tested backcross population (cytolines)also under well-watered and water-limited conditions in a nethouse experiment. In the cytolines, we found variations in the response to the different abiotic stresses for the fitness traits and in clock period and especially for amplitude. In the BC1S4, most of the lines were less affected under high temperatures as compared to the cultivar for fitness traits. The CMPP population is still new but we already found differences in Days to Flowering between carriers of the wild and cultivar plasmotypes among several CMPP sub-families in the first field propagation. These findings derived from the Barley1K framework pave the way to unravel genetic networks and mechanisms underlying plant robustness and their role in plant adaptation and evolution. The CMPP will also enable testing of the cytonuclear interactions between the chloroplast and nuclear genome and will pave the way to utilize this neglected diversity in barley breeding for different environments.

KEYWORDS: Plasmotype, Circadian clock, Wild barley

WILD BARLEY RE-DOMESTICATION: MAKING WILD BARLEY DWARF AND POWDERY MILDEW RESISTANT WITH CRISPR/CAS9 GENOME EDITING

Jovana Janjić
Department of Agroecology – Crop Genetics and Biotechnology, Aarhus University, Forsøgsvej 1, 4200 Slagelse, Denmark

jovana@agro.au.dk

Co-Authors:

Tobias Hanak, Claus Krogh Madsen, Henrik Brinch-Pedersen
Department of Agroecology – Crop Genetics and Biotechnology, Aarhus University, Forsøgsvej 1, 4200 Slagelse, Denmark

Wild ancestors of modern crops can be adapted to various unfavorable environmental conditions, and their genetic diversity can be a valuable resource for solving challenges in agriculture caused by climate changes. However, identification and transferring of genes responsible for the adaptations to elite cultivars is not highly successful. Re-domestication of wild ancestors using novel breeding techniques, such as CRISPR/Cas9 mediated genome editing can be significantly faster and a more successful approach. The approach enables precise mutations in domestication genes while retaining beneficial traits of the wild ancestors. The aim of the current study is to introduce two secondary domestication traits in wild barley – dwarfism and powdery mildew resistance. To reach these aims, we will knock out the dwarfing gene HvDep1 and the Mlo locus, conferring resistance to powdery mildew. For achieving this, we use both simplex and multiplex editing strategies. pANIC6A vector is used for simplex and pTRANSv240 for multiplex genome editing with four sgRNAs (two targeting each gene). To validate the success of designed constructs, we are currently transforming the non-dwarf barley cultivar "Maythorpe" via Agrobacterium-mediated transformation of immature embryos. So far, we were able to induce shooting and rooting in tissue culture after transformation and selection. Moreover, the HvDep1 were sequenced in 10 different wild barley lines. The coding sequence of the gene is highly conserved (>99 % sequence identity between cultivars and wild barley lines). Sequencing of the Mlo from wild lines is currently ongoing. Results from all mentioned experiments will give us the necessary basis for proceeding with wild barley transformation and re-domestication.

Aknownledgements: This research was funded by Novo Nordisk Foundation Challenge grant 'NOVOCROPS', NNF19OC0056580.

KEYWORDS: genome editing, CRISPR/Cas9, barley, dwarf, powdery mildew

BARLEY BREEDING: SUCCESS STORIES

BARLEY CULTIVAR DEVELOPMENT IN EUROPE – SUCCESS IN THE PAST AND POSSIBLE CHANGES IN THE FUTURE

Birger Eriksen
Sejet Plant Breeding I/S, Denmark
bee@sejet.dk

SUCCESS IN THE PAST.

The first barley genetics symposium that I attended after starting as a barley breeder in 1987, was the IBGS-6 in Helsingborg where Gerhard Fischbeck made a presentation with the same title as the above. His introduction to barley breeding made a big impression, and for me put things in a historical perspective. In the presentation, Fischbeck explained how modern breeding started with selection in landraces in a few well-defined regions in Europe, with subsequent inter crossing of the original landrace varieties (1920-30s). In the second round of crossbreeding, the combination of genetics from different regions resulted in important varieties, such as Bonus and Ingrid, both created by combining varieties from Moravia and Sweden. The further introduction of new genetic resources and mutation breeding completed the steps towards modern varieties like Trumpf and Alexis. The present presentation subsequently describes the ongoing history of variety development in spring barley by looking at the largest varieties for the last 15 years and by looking at pedigree trees and varieties most widely used as crossing parents.

SUCCESS IN THE PRESENT.

Success for a crop species is not just a matter of performance of individual varieties but is just as much about the economic capacity and relevance of the crop. With examples from Denmark, I will look at the acreage and yields of spring and winter barley in relation to the other cereal species and give examples and explanations of the shift in importance. For barley, the supply to the malt, brewing and distilling industry is of great importance, and the development in malting quality has been and is of invaluable importance to the industry.

SUCCESS IN THE FUTURE.

Looking into the future, there is one omnipresent trend which is the green transition into more climate neutral production. A very concrete example of the changing of policies in this context is EU's "Green Deal" and "Farm to Fork". Unfortunately, crop production is a major contributor to the world's emissions of greenhouse gases, and the CO2-footprint for all food and feed products will be high on the agenda, and plant breeding will undoubtedly get a prominent position in the green transition. In the future, there will thus be a completely new look at the efficiency of new varieties (VCU-sustainability). In focus will be yield, yield stability, decreasing inputs, nutrient efficiency, and stress tolerance. Also very important will be the quality of our cereals as raw material for industry process for further improving the climate profile of products. Part of the "Farm to Fork" strategy is to halve the use of pesticides meaning that the genetic disease resistance must be significantly strengthened to avoid losses in the field. Deregulation of NGT (e.g. CRISPR) will surely ease the fulfillment of the above goals.

KEYWORDS: Barley breeding history, important spring barley varieties, future breeding goals, green transition

NEW DEVELOPMENTS IN IP IN PLANT BREEDING – A GOLDEN OR A DARK AGE FOR INNOVATION?

Alexander Strube
Ackermann Saatzucht GmbH & Co. KG
strube.alexander@sz-ackermann.de

For decades, progress in both commercial and scientific plant breeding has been driven by the breeder's exemption entitling breeders and researchers around the world to work to access any commercially released germplasm for crossing. Without a doubt, this open access to germplasm has been providing breeders with a safe and transparent legal framework with almost no transaction costs for obtaining such access.
However, particularly with the advent of gene-editing, the protection of technologies, processes and traits by patents is expected to rise in the coming years, at a time when a quick and broad uptake of innovations is particularly in demand. The presentation will explore the benefits and disadvantages of each innovation system as well as several potential avenues to handle the disparate systems of the plant breeder's rights vs. patenting. Ultimately a strong case must be made for enabling the breeding industry as a whole to maintain its diversity, with efficient and collaborative ways to bring solutions for sustainable agriculture and food safety as quickly as possible.

Aknownledgements: Bill Thomas, James Hutton Institute

KEYWORDS: breeder's exemption, patent, IP, crossing agreement

BREEDING FOR THE PRODUCIBILITY OF FEMALE PARENTAL LINES IN HYBRID BARLEY

Emmanuelle Dyrszka
SYNGENTA
emmanuelle.dyrszka@syngenta.com

Co-Authors:

Pauline Marty, Jan Gielen, Alix Tanoh-Gory, Edouard Tatara, James Melichar
SYNGENTA

Hybrid barley is part of the innovative solutions that Syngenta developed to deliver high-yielding varieties to the farmers. As for any hybrid crop, the 'cost-of-goods' related to the hybrid seed production is an essential parameter and therefore represents a key target during the breeding process.
Since the very first introduction of hybrid barley varieties, Syngenta has been exploring different genetic approaches to improve the producibility of our parental lines, notably of the male-sterile female lines. GWAS analysis on seed set from historical data allowed for the identification of a major QTL for producibility in the female gene pool. The effect of the QTL is currently validated by introgressing the favorable allele in female lines with poor producibility. Since seed set is well-known to represent a quantitative trait and therefore involves many more regions of the genome, we also developed a genomic selection model to predict the producibility of potential new female lines. The QTL and the genomic prediction model now are fully deployed during the early breeding stages to support the development of female lines that meet the requirement for producibility. In addition to the selection for seed set, we also characterize the allelic diversity at the Rfm3 locus to reduce the incidence of partial male fertility as described by Bernhard et al. (2019). The selection for superior sterile alleles at the Rfm3 locus further improved the breeding efficiency in the female pool.
In all, the integrative approach of combining molecular tools with predictive models to support our breeding efforts for enhanced producibility of the female parental lines has further improved the competitive advantage of barley hybrids in the marketplace.

KEYWORDS: Hybrid barley, producibility, seed set, partial male fertility

PUTTING MILLIONS OF MARKERS AND THOUSANDS OF YIELD PLOTS TO THE TEST: GENOMIC PREDICTION OF 2-ROW SPRING BARLEY

Pernille Sarup
Nordic Seed, Denmark
pesa@nordicseed.com

Co-Authors:

Jens Due Jensen, Jihad Orabi, Ahmed Jahoor
Nordic Seed, Denmark

Genomic prediction can be used to do early selection at a generation stage where we do not yet have enough seeds to produce reliable and reproducible phenotypes in the field for complex traits such as yield or malting quality. Prediction in F5 dramatically increases selection intensity and reduces generation time, two important components determining genetic gain from selection.
The increase in genetic gain from implementing genomic prediction can be limited by the accuracy of the prediction. Accuracies of genomic prediction in part depend on some population and trait given characteristics, such as plot heritability and genomic relationship between the training population and the lines we want to predict. Accuracies of the genomic prediction also depend on factors breeders can more easily change, such as the number of markers, plots, locations and years we use in the training population. Here we present results from genomic prediction in a population of 3145 2-row spring barley breeding lines, each genotyped for 7K segregating markers and tested in blocks across nine years in a total of 37K plots distributed over 634 environments.

KEYWORDS: Genomic Selection

A CENTURY OF BARLEY RESEARCH AND BREEDING IN BRAZIL

Euclydes Minella
EMI Consultoria Ltda. Rua Pássaros e Flores, 223/106 São Paulo, SP Brazil cep 04704
allenim54@yahoo.com.br

Barley is poorly adapted to the acid, aluminum toxic soils and warm wet weather conditions, predominant in most sub-tropical/tropical areas of the world. Tailoring the crop to fit these adverse conditions has challenged scientists in Brazil over a century now being1920, the onset of barley research and breeding. Increasing beer consumption has pushed domestic malt and malting barley production since early 1930's. Production until late 1960 was mainly in non-acid soil, deforested lands of southern Brazil, with imported varieties from Argentina and Europe. Selection of varieties with some degree of soil acidity/aluminum toxicity tolerance was the first major achievement in breeding, allowing the expansion of the crop production in the already mechanized lands limed soil areas. The release of early, net blotch resistant, high yielding variety "Cevada BR 2" in 1990, was a cornerstone in the consolidation of a malting barley industry. The widespread adoption of the "No Tillage" in 1990's, in the double cropping grain production system by farmers, represent the third major achievement that significantly improved the yield potential and consequently, the competitiveness of barley compared to wheat, the major competitor. The increased soil production capacity under the "No-Till Production System", demanded for varieties more adapted to this technology. The release of the disease and lodging resistant, short strawed, high yielding varieties BRS 195 (2000), BRS Cauê (2006) and BRS Brau (2010) by Embrapa, was the fourth major event in the crop success history, revolutionizing barley production. The widespread use of these improved genotypes has boosted yield and malting quality, making barley even a more competitive crop in this century. Since then, farm productivities over 6,000 kg/ha have been harvested in favorable seasons, increasing the average farm yield from 1,500 kg/ha in the 1980's to 3,500 kg/ha in the 2000's. Varieties BRS 180 (1999), BRS 195 (2000), BRS Sampa (2009), BRS Manduri (2011) and BRS Itanema, made barley production feasible also in the more tropical lands of the Southeast and Central west (Cerrado) regions, under irrigation, where yields over 7,000 kg/ha are not difficult to obtain. However, the breakthroughs in yield, quality and disease resistance have not reduced to a satisfactory level yet, the production/quality instability due to excessive rainfall during harvesting, particularly in ENSO years, in southern Brazil. On the average, the volume of harvested grain that does not meet quality for malt is around to 20% yiearly. Besides pre-harvesting sprouting, increased losses to Fusarium Head Blight and/or DON contamination, possibly associated with the no till practice, are frequent, being the major challenge for barley scientists in this new century, concerning the sustainability of the malting barley industry.

KEYWORDS: Barley, tropical, No Till, breeding, soil acidity

SUCCESS IN BREEDING FOR BYMOVIRUS RESISTANCE BARLEY VARIETIES - ACHIEVING THE SAME GOAL WITH DIFFERENT STRATEGIES IN EUROPE AND CHINA

Ping Yang
Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China
yangping@caas.cn

Co-Authors:

Congcong Jiang, Jing Zhan
Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China

Huiquan Shen
Jiangsu Coastal Area Institute of Agricultural Sciences, Yancheng, China

Rugen Xu
Colleague of Agronomy, Yangzhou University, Yangzhou, China

The barley yellow mosaic virus disease was a disaster for autumn-sowing barley cultivation in Europe and East Asia during the fourth quarter of last century. This disease is caused by individual or combined infection of barley yellow mosaic virus (BaYMV) and barley mild mosaic virus (BaMMV) via the transmission by soil-borne plasmodiophorid Polymyxa graminis. Breeding for varieties carrying resistance gene(s) has been approved as the optimal strategy in disease control.
In Europe, the application of agriculturally-important resistance loci rym4 or rym5 lead to a success in breeding for virus-free (complete resistance) varieties, despite several resistance-breaking strains were reported. In China where the resistance-conferring alleles of two host factor genes HvEIF4E and HvPDIL5-1 are over-represented in population of Chinese landraces, breeding for medium to high resistance (disease symptom is absent or mild, but virus particles are mostly detectable by ELISA or RT-PCR) was conducted in the breeding programs since 1980s.
The difference on the breeding strategy in Europe and China are likely due to: (1) complexity of local virus populations, (2) application of disease severity quantification methods, as well as (3) difference in local climates. Now the resistance loci rym4, rym5 as well as rym1/11 are infected but still show levels of resistance in the fields of China, where barley breeding success largely relies on rym5 as well as two newly-identified resistance loci rym2H-1 and rym7H-1.

KEYWORDS: Bymovirus, BaMMV/BaYMV, Resistance breeding, Host factors

RE-DOMESTICATING BARLEY: A SMALL STEP FOR GENETICS AND A GIANT LEAP FOR BARLEY BREEDING

Duane Falk
Professor Emeritus, Department of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada
dfalk@uoguelph.ca

Co-Author:

Chris Grainger
CEO, Harvest Genomics Inc., Guelph, Ontario, Canada

Barley (H. vulgare sub vulgare) was domesticated from wild barley (H. vulgare sub spontaneum) in the Middle East about 10,000 years ago. The major domestication trait, loss of the brittle rachis, has been traced to two different loss-of-function deletions, Btr1>btr1 and Btr2>btr2, on barley chromosome 3H. The majority of modern barley genotypes trace back, ultimately, to likely one or two homozygous, original founder individuals. The variation found in modern domestic barley landraces and cultivars has come from subsequent spontaneous mutation, introgression from wild barley populations, and outcrossing among domestic. Modern breeding has led to erosion of genetic. It is recognized that there is a largely untapped pool of potentially useful diversity in wild barley populations. In light of changing climate and the evolution of pathogens to greater virulence and aggressiveness, it is highly desirable to be able to access the deep gene pool of wild barley.

We are introgressing the basic domestication trait of non-brittle rachis into a number of populations of diverse wild barley. Efficient allele-specific DNA markers have been developed for both the btr1 and btr2 non-brittle rachis alleles that make their introgression into wild barley a relatively simple and efficient process. Introgressing the desirable btr alleles is being facilitated by the use of a xenia-expressing, morphologically-marked male sterile gene system (sex1-o-msg6). A set of 15 wild barleys representing a range of geographic regions from Libya to Kazakhstan, from below sea level to over 2000m above sea level has been assembled. Each line has been crossed with male sterile barley lines in several diverse 2R and 6R backgrounds, including some hulless materials. The F1 and F2 generations have been grown and putative non-brittle rachis plants identified phenotypically. Selected plants are being verified with allele-specific DNA markers. Their male sterile progeny will be backcrossed to the recurrent wild barley parent to generate the BC1 population. The process will be repeated for another cycle to produce non-brittle BC2 populations (87% wild barley) which will be evaluated for numerous traits. Desirable lines segregating for the marked male sterile gene will be released to breeders, as well as intercrossed among the various wild barley background populations. A relatively simple process for modern genetics is being used to open up a huge new source of genetic diversity for modern barley breeding.

KEYWORDS: non-brittle rachis, wild barley, introgression, diversity

GENOTYPING OF BARLEY USING OPTIMIZED SNP ARRAYS

Joerg Plieske
ISGS Institut Fresenius GmbH TraitGenetics Section (SGS IF TG), Am Schwabeplan 1b, 06466 Seeland OT Gatersleben, Germany
joerg.plieske@sgs.com

Co-Authors:

Heike Gnad, Andreas Polley, Dagmar Kulosa, Thomas Gross, Martin W. Ganal
SGS Institut Fresenius GmbH TraitGenetics Section (SGS IF TG), Am Schwabeplan 1b, 06466 Seeland OT Gatersleben, Germany

Genotyping of barley for plant genetic studies, germplasm and variety characterization, research and plant breeding requires a compromise between accuracy, resolution and costs per sample. In order to develop such optimized genotyping arrays for barley, we have assembled public SNP marker data sets mainly based on results from barley genotyping using the published 50K array (Bayer et al. 2017) that (i) comprise of molecular markers that are of high technical quality, (ii) display a good level of polymorphism over a wide range of breeding, adapted and wild material based on hundreds of genotyped lines; (iii) are not in perfect linkage disequilibrium with each other; (iv) are evenly distributed along the chromosomes based on genetic recombination; and (v) are markers reported to be linked to specific traits. Especially, for plant breeding purposes such as Genomic Selection and marker-assisted breeding, we have developed an optimized 15K barley genotyping array and a smaller 4K barley genotyping array that can be used together with marker imputation using data from larger arrays (barley 50K array) or genome sequencing.
These optimized genotyping arrays for barley are now being used widely within genetic research and accelerated plant breeding both for the academic and private sector.

KEYWORDS: Barley, molecular marker, SNP marker, micro arrays

GENETICS AND BREEDING OF BIOMASS (RESOURCES USE EFFICIENCY/RUE)

PHYSIOLOGICAL TRAITS ASSOCIATED WITH YIELD PROGRESS IN COMMERCIAL MALTING BARLEY GENOTYPES RELEASED FROM 1980 TO 2019 IN ARGENTINA

Daniel Miralles
University of Buenos Aires, School of Agriculture and CONICET, IFEVA
miralles@agro.uba.ar

Co-Authors:

Victor Gimenez, Nicolas Ciancio
University of Buenos Aires School of Agriculture

Leonor Gabriela Abeledo
University of Buenos Aires, School of Agriculture and CONICET, IFEVA

Barley is the fourth most important cereal in the world with 7% of the area cultivated with cereals (FAOSTAT, 2022). About 70%–85% of barley is used for animal feed and 15%–20% is destined for malting (Fischbeck, 2001). In Argentina, barley is mostly used for malting being the most important supplier of malt for the countries of South America. Harvested area of malting barley in Argentina increased at a rate of 57.600 ha y-1 from 1990s, reaching 1.2 million hectares in 2021. Yield at national level was increased from the ´80s at a rate of 65 kg ha y-1 with an actual production of 5 million Tons. To determine the i) genetic improvement on yield potential and ii) the physiological attributes associated with yield of malting barley in Argentina under no restrictive water and nutritional conditions, field experiments were carried out at the School of Agriculture of Buenos Aires (FAUBA) during 2020 and 2021 growing seasons. Eleven representative commercial barley cultivars released in the Argentine market from 1982 to 2019 were analyzed. Cultivars were arranged in a completely randomized design with four replications in plots of 6,125 m2. Crop density was 270 pl m-2. Results showed that duration of the cycle from emergence (Em) to physiological maturity (PM), measured in thermal time (ºCd), increased slightly but significantly (3 ºCd y-1; $p < 0.05$). Similar trend was observed for the period Em-Anthesis (An). However, grain filling period was not significantly modified by breeding with an average of 400 ºCd (base temperature = 8 ºC). Grain yield increased at a rate of 69 kg ha-1 y-1 (from 6 to 9 Tons ha-1) due to an increase in grain number per unit area (77 grains m-2 y-1) as well as in average grain weight (0.22 mg y-1). Aboveground biomass was also significantly increased ($p<0.05$) by breeding at a rate of 64 kg ha-1 y-1. The same trend was observed in harvest index that was increased from 0.4 to 0.5 representing a rate of 0.0023 y-1. The physiological attributes of biomass (Cumulative Intercepted Radiation -Radcum- and Radiation use efficiency -RUE-) during the cycle were also analyzed. Radcum during the whole cycle from Em to PM was only increased in 2020 but not in 2021. Thus, in 2020 both Radcum during pre and post flowering periods were increased by breeding. RUE during the whole cycle (Em-PM) was not modified by breeding in any of the growing seasons because of different and opposite trend during pre and post flowering period (especially in 2020). Thus, pre-flowering RUE in 2020 decreased with the year of release at a rate of -0.021 g MJ-1 m-2 y-1, while post flowering RUE was slightly increased at a rate of 0.0105 g MJ-1 m-2 y-1. In summary, breeders increased yield during the last 40 years due to increases in grain number and grain weight. Regarding the physiological yield components, yield gains were associated with both aboveground biomass and harvest index. However, RUE was not modified by breeding.

KEYWORDS: Barley breeding, Biomass, RUE, Yield

PHYSIOLOGICAL AND AGRONOMICAL ASPECTS OF POTASSIUM USE EFFICIENCY (KUE) IN BARLEY

Lana Shabala
Tasmanian Institute of Agriculture, College of Science and Engineering, University of Tasmania, Hobart, Tas 7005, Australia; International Research Centre for Environmental Membrane Biology, FoshanUniversity, Foshan 52800

l.shabala@utas.edu.au

Co-Authors:

Widad Al Azzawi, Meixue Zhou, Tina Acuña
Tasmanian Institute of Agriculture, College of Science and Engineering, University of Tasmania, Hobart, Tas 7005, Australia

Muhammad Bilal Gill
International Research Centre for Environmental Membrane Biology, Foshan University, Foshan 52800; Tasmanian Institute of Agriculture, College of Science and Engineering, University of Tasmania,Hobart, Tas 7005, Australia0, China

Sergey Shabala
Tasmanian Institute of Agriculture, College of Science and Engineering, University of Tasmania, Hobart, Tas 7005, Australia; International Research Centre for Environmental Membrane Biology, FoshanUniversity, Foshan 52800

Potassium deficiency is one of the major issues affecting crop production around the globe. Giving the high cost of potassium fertilizers and environmental concerns related to inappropriate fertilization practices, developing more potassium use efficient (KUE) varieties is critical for sustainable food production in agricultural systems. In this study, we analysed impact of potassium availability on agronomical attributes of thirty barley genotypes grown under glasshouse conditions at various potassium supply (0.002 mM, 0.02 mM, 2 mM, 20 mM). The results showed that the availability of potassium in the soil had a major effect on yield components i.e., spike number, grain number and grain weight. Furthermore, grain weight showed a strong correlation with grain number and spike number at all levels of potassium supply. Although an increase in potassium supply led to an increase in plant height in all genotypes, the correlation with grain weight was very weak at all levels. Potassium supplementation caused an increase in shoot dry weight, which also showed a weak correlation with grain weight at the majority of potassium supply levels. Cluster analysis based on low K (0.002mM) availability resulted in four genotype groups, with major differences being for leaf K content, xylem Na content, and stomatal conductance. A significant negative correlation between leaf K and grain yield was observed under intermediate levels of K availability (0.02 and 2 mM). Grain yield showed positive correlation with the xylem K concentration at all K levels except the highest (20 mM). The positive correlation suggests that plants remobilise K to the grains to produce more yield, which is a crucial mechanism for KUE in plants. Under the highest K availability a negative correlation was found between grain yield and xylem K, most likely as a result of reduced uptake of magnesium (Mg) and calcium (Ca) uptake. The highest K treatments (2 and 20 mM) resulted in a positive correlation between grain yield and Na concentration in leaf and xylem. Overall, our work revealed several genotypes that were highly efficient in performing at suboptimal K supply levels and, thus, can be recommended to be grown in K-impoverished soils. We also suggest that grain and spike numbers could be used as proxies for KUE studies, to construct DH lines and identify QTL to improve low potassium tolerance and KUE in barley

KEYWORDS: Barley genotypes, crop production , potassium use efficiency (KUE), mechanism for KUE, correlation with yeald

THE BARLEY MUTANT HAPPY UNDER THE SUN 1 (HUS1): AN ADDITIONAL CONTRIBUTION TO PALE GREEN CROPS

Paolo Pesaresi
Department of Biosciences, University of Milan, I-20133 Milan, Italy
paolo.pesaresi@unimi.it

Co-Authors:

Lisa Rotaspertia, Luca Tadini, Matteo Chiara, Andrea Tagliani, Sara Forlani, Ignacio Ezquer, David S Horner
Department of Biosciences, University of Milan, I-20133 Milan, Italy

Cristina Crosatti, Davide Guerra, Alessandro Tondelli
CREA – Research Centre for Genomics and Bioinformatics, I-29017 Fiorenzuola d'Arda, Italy

Peter Jahns
Plant Biochemistry, Heinrich Heine University, D-40225 Düsseldorf, Germany

Katarzyna Gajek, Agnieszka Janiak
Institute of Biology, Biotechnology and Environmental Protection, University of Silesia in Katowice, P-40–032 Katowice, Poland

Addy Garcia, Roxana Savin
Department of Crop and Forest Sciences, University of Lleida - AGROTECNIO Center, Av. R. Roure 191, S-25198 Lleida, Spain

Laura Rossini
Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy, University of Milan, I-20133 Milan, Italy

Truncated antenna size of photosystems and lower leaf chlorophyll content has been shown to increase photosynthetic efficiency and biomass accumulation in microalgae, cyanobacteria and higher plants grown under high-density cultivation conditions. Here, we have asked whether this strategy is also applicable to a major crop by characterizing the barley mutant happy under the sun 1 (hus1). The pale green phenotype of hus1 is due to a 50% reduction in the chlorophyll content of leaves, owing to a premature stop codon in the HvcpSRP43 gene for the 43-kDa chloroplast Signal Recognition Particle (cpSRP43). The HvcpSRP43 protein is responsible for the uploading of photosystem antenna proteins into the thylakoid membranes, and its truncation results in a smaller photosystem antenna size. Besides a detailed molecular and physiological characterization of the mutant grown under controlled greenhouse conditions, we show that the agronomic performance of hus1 plants, in terms of total biomass production and grain yield under standard field conditions, is comparable to that of control plants. The results are discussed in terms of the potential benefits of the hus1 phenotype, and of natural allelic variants of the HvcpSRP43 locus, with respect to productivity and mitigation of climate change.

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We are also grateful to MIUR-PRIN 2017FBS8YN to PP.

KEYWORDS: Photosystem antenna size, Pale green leaves, Photosynthesis efficiency, Hordeum vulgare, Arabidopsis thaliana

DIVING INTO THE GENETIC DIVERSITY OF THE EUROPEAN HERITAGE BARLEY COLLECTION (EXHIBIT)

Sonia Negrao
School of Biology and Environmental Science, University College Dublin, Dublin, Ireland
sonia.negrao@ucd.ie

Co-Authors:

Villő Bernád, Nadia Al-Tamimi, Patrick Langan, Tim Dempsey, Joey Henchy, Mortaza Khodaeiaminjan
School of Biology and Environmental Science, University College Dublin, Dublin, Ireland

Gary Gillespie, Mary Harty, Kevin P. Mcdonnel
Crop Sciences, School of Agriculture and Food Science, University College Dublin, Dublin, Ireland

Luke Ramsay, Kelly Houston, Joanne Russell, Robbie Waugh
The James Hutton Institute, Dundee, UK

In Ireland, spring barley is the most cultivated cereal, and production must meet an increased demand of a flourishing malting industry. Modern barley cultivars have been bred under optimal conditions, leading to a reduced stress tolerance. In contrast, robust stress tolerance can be found in heritage germplasm. Here we explore the European HerItage Barley collecTion (ExHIBiT), a collection comprising 363 two-row spring-barley accessions originating from across Europe, including landraces, formerly bred cultivars and modern cultivars, which has been assembled from different germplasm sources/projects. The ExHIBiT collection was genotyped with the 50K SNP array and showed a population structure with four distinct groups. A power analysis established an association mapping population of 230 accessions that have been phenotyped in the field in Ireland for two years, and that can be used to identify loci contributing to stress tolerance, disease resistance or other agronomic traits.
Climate change is causing extreme weather events such as flooding and droughts. Barley is the most susceptible cereal to waterlogging with yield losses of approximately 20-25% and improving waterlogging tolerance has been flagged as a major goal for future breeding programs. To investigate waterlogging tolerance, the ExHIBiT collection is being phenotyped in controlled conditions using RGB, chlorophyll fluorescence and hyperspectral sensors as well as in field conditions using Unmanned Aerial Vehicles (UAV) coupled with RGB and multispectral sensors. The most recent data will be presented.

Aknownledgements: This research was supported by Science Foundation Ireland Centre by the SFI President of Ireland Future Research Leaders to SN under Grant No.18/FRL/6197.

KEYWORDS: Heritage barley, image-based phenotyping, waterlogging

BARLEY END USES: FROM FOOD AND FEED TO MALTING, BREWING, AND NOVEL PRODUCTS

NEW POTENTIALS OF THE BARLEY GRAIN

Henrik Brinch-Pedersen
Aarhus University, Department of Agroecology, Research Center Flakkebjerg, Slagelse, Denmark
hbp@agro.au.dk

Barley is a robust plant able to grow in different environments, including marginal areas and high altitudes. In addition to this, it has many non-utilized potentials within food, feed and industrial products. Barley has significant potentials in the green transition and in a climate challenged future.
A range of studies suggests how the nutritional value of barley can be improved significantly. The digestibility of major dietary components can be accelerated through targeted improvements of the grain. Modulation of the mature grain phytase activity constitutes a potent handle for improving phosphate and micronutrient bio-availability in food and feed. Moreover, grain traits supporting efficient digestion of protein, phytic acid and fibre by exogenous feed enzymes have been identified.
Targeted gene knockouts and accelerated gene expression can efficiently be generated in barley by modern tilling approaches and New Breeding Techniques. Techniques like these facilitates new developments of barley for agricultural and industrial uses and exploitation of resilient wild barley through re-domestication.

KEYWORDS: Barley, Food, Feed, Industrial Products, New Breeding Techniques

ACCELERATION WITH A PURPOSE – ULTRAFAST BARLEY TRAIT DEVELOPMENT FOR SUSTAINABLE BEER PRODUCTION

Christoph Dockter
Carlsberg Research Laboratory, J.C. Jacobsens Gade 4, DK-1799 Copenhagen V, Denmark
christoph.dockter@carlsberg.com

At a global scale, food security is challenged by crop productivity stagnations that are aggravated by man-made climate change. This climate emergency calls for low carbon footprint solutions across the brewing industry, including a boost of sustainable crop improvement through new, targeted genome engineering technologies and their immediate application. Here we present 'FIND-IT', a fast and high-throughput strategy to isolate novel traits for the development of future raw materials - climate-resilient and suitable of cutting carbon footprints within the brewing value chain.
This simple and agile approach combines systematic sample pooling-and-splitting with high-sensitivity genotyping to screen extremely large, low mutation-density variant populations for targeted identification of desired traits. The strength of FIND-IT is demonstrated by targeted isolation of knockouts, missense, miRNA and promoter variants in libraries of elite barley (Hordeum vulgare) cultivars. Low mutation rates per individual make FIND-IT variants directly applicable to elite breeding pipelines, and minimize time-consuming technical steps thereby accelerating barley evolution towards sustainable beer production.

KEYWORDS: Beer, barley, breeding, carbon footprint, climate change

ASSESSMENT OF WINTER BARLEY FOR FEEDING QUALITY TRAITS TO BREED FOR IMPROVED PROTEIN UTILIZATION IN PIG NUTRITION

Markus Herz
Bavarian State Research Center for Agriculture (LfL), Institute für Crop Science, Barley Breeding Group
Am Gereuth 6, D-85354 Freising

markus.herz@lfl.bayern.de

Co-Authors:

Jennifer Groth, Wouter Vahl, Stephan Schneider, Martin Schaeffler
Bavarian State Research Center for Agriculture (LfL), Institute für Crop Science, Barley Breeding Group
Am Gereuth 6, D-85354 Freising

Knowledge was gained about the possibility to optimize amino acid composition of winter barley by breeding and thus to increase nutrient efficiency in pig farming. Grain samples from official variety trials were examined for feed quality traits. Furthermore a winter barley collection consisting of 80 varieties of different origin was tested in field- and greenhouse under different nitrogen fertilization levels and examined for amino acid composition. Experiments were carried out in an automated greenhouse that allows to perform experiments under controlled environmental conditions and to record growth dynamics above and below ground using automated imaging. Analysis of grain samples allowed to quantify variation between varieties as well as between the effects of N fertilization on forage quality. Differences between varieties were observed in the range of up to 10% for lysine concentration and as much as 60% for phosphorus content. Influence of genotype and nitrogen fertilization respectively on protein quality was reproducible across the experiments. Several winter barley varieties could be identified showing the desired range of values for several traits.
A genome-wide association study using the 50k iSelect SNP chip yielded numerous marker-trait associations with significant effects on forage quality as well as grain yield. Most of the significant markers were located on chromosomes 5H and 6H, a smaller number on chromosomes 1H and 7H. There were markers that only had an effect on a single trait and those that simultaneously influenced several traits in the desired direction.
The collected phenotypic and genetic data form a solid basis to breed for improved forage quality in winter barley, to reduce fertilizer and nutrient leaching and to optimize the nutrient cycle in pig feeding from field to barn. After an evaluation of the marker- information marker assisted selection for protein quality is expected to be applied successfully in breeding programmes.

KEYWORDS: winter barley, feed quality, amino acids, nutrient efficiency

MOLECULAR MARKERS FOR MALTING BARLEY BREEDING FOR PGI CZECH BEER

Leona Leišová-Svobodová
Crop Research Institute
leisova@vurv.cz

Co-Authors:

Pavel Svoboda, Zdeněk Nesvadba
Crop Research Institute

Barley is one of the most important crops in the world. Barley is used as food and feed and is important for malting. Demands for malt quality vary between countries and customs. Malting quality is a complex characteristic including barley genetics, environmental conditions during barley growth and technological parameters of the malting process. In this study, it was hypothesized that there are no differences between the two groups of barley varieties with different but defined malting qualities.
Testing was performed by transcriptome sequencing at selected stages of malting. A total of 919 differently transcribed genes between the two groups of barley were identified and annotated.
The most important differences were found in the ontological groups of membrane components, including transmembrane transport, redox processes and genes involved in hydrolase activity. Sequence differences (SNPs, INDELs) were used for primer designs. After optimization and validation, three molecular markers were developed for use in barley breeding.

Aknownledgements: Finantial support MZe-QK1910197 and MZe-RO0418.

KEYWORDS: Hordeum vulgare, malting quality, transcriptom, Ilumina, MAS

MEDWHEALTH: HIGH AMYLOSE DURUM WHEAT AND A RECENTLY NAKED HIGH B-GLUCAN BARLEY VARIETY TO PRODUCE HEALTHIER TRADITIONAL FOOD REPRESENTING MEDITERRANEAN HERITAGE

Andrea Visioni
International Center for Agricultural Research in the Dry Areas (ICARDA)
a.visioni@cgiar.org

Co-Authors:

Abderrazek Jilal
Plant Breeding Department, Institute of Agronomic Research of Morocco. Rabat, Morocco

Hajer Ben Ghanem
Field Crops Laboratory, Institute of Agronomic Research of Tunisia. Tunis, Tunisia

Benbelkacem Abdelkader
Plant breeding and Biotechnology Unit, Institute of Agronomic Research of Algeria. Constantine, Algeria

Rola El Amil
Plant Breeding Department, Lebanon Agricultural Research Institute. Tal Amara, Lebanon

Dina Najjar
Resilient Agri-Food System, International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco

Houda Bousselham
International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat Morocco.
Universite des Sciences et Technique Hassan II, Mohammedia (Maroc)

Francesco Sestili
Department of Agriculture and Forestry Sciences (DAFNE), University of Tuscia, Viterbo (Italy)

MEDWHEALTH project focuses on improving the Mediterranean Diet, a diet-based lifestyle spread in Mediterranean areas, recognized as useful in the health maintenance and diseases prevention. MEDWHEALTH (awarded PRIMA project in 2020) aims at re-designing a selection of typical Med-Foods valorizing their healthiness with a set of valuable and innovative raw materials, including durum wheat (high amylose, cv. Svevo HA), barley (high β-glucans, cv. Chifaa) and lentil (high protein content, ICARDA elite line) and related processing. Pasta, couscous, bulgur, freekeh, bread, traditional snacks (sweet and salted) along with a pool of niche-regional foods will be produced by using an innovative and more healthy semolina containing elevated amount of resistant starch, alone or blended with other highly nutritious flours from barley and lentils (SDG 2, zero hunger; SDG 3, good health and well-being). Barley has almost virtually disappeared from the solid diet in many countries and is mostly used as animal feed and/or for malt. However, it remains an important crop in many countries in the MENA region and is recently gaining importance as healthy food in many developed countries due to its important nutritional features such as β-glucans and antioxidant compounds. β-glucans reduce blood cholesterol concentration as well as increase satiety reducing energy intake and postprandial glycemic response and improving digestive function. β-glucans are also immunostimulants against infectious diseases and some types of cancers. Chifaa is a huskless barley variety recently released by INRA Morocco, it is characterized by high protein and high β-glucans, resistant or moderately resistant to diseases and drought tolerant. Huskless barley,

do not require mechanical pearling thus simplifying processing and reducing workload, costs, energy and as well as the loss of 15-20% of grain content during the pearling. Furthermore, after harvest the grain is already in proper shape to do the small-scale processing for couscous, bakery and bread etc. With a view to preserving traditions, female cooperatives and SMEs of different countries will be engaged to realize local food products using raw materials proposed in the project. The new/old Med-Foods will be fully characterized for health-related components and their actual benefits on humans, as well as for market acceptance and economic value. UNITUS and ICARDA, in synergy with the National Research Centers of the North African (Morocco, Algeria, Tunisia) and Mid-East area (Lebanon), embark on developing and promoting these foods by working closely with local women cooperatives and SMEs operating in the food sector, providing them with the improved raw materials and the development of novel recipes developed in the project. This will contribute to enhancing women's cooperatives bargaining power in the market, improving women's income, and working conditions (SDG 5, gender equality, and SDG 8, decent work, and economic growth).

Aknownledgements: We acknowledge the PRIMA foundation for supporting our project in the frame of Section 1 Call 2020 - Agro-Food Value Chain.

KEYWORDS: Chifaa, huskless barley, food, Med-Diet, β-glucans, women cooperatives, lentil, high amylose durum wheat, Svevo HA

COMPARATIVE EVALUATION OF THE GRAIN CHEMICAL COMPOSITION OF HULLESS BARLEY VARIETIES

Mara Bleidere
Institute of Agricultural Resources and Economics
mara.bleidere@arei.lv

Co-Authors:

Vita Šterna, Ilze Grunte
Institute of Agricultural Resources and Economics

Hulless barley (HB, Hordeum vulgare L.) research and development is receiving more emphasis with potential for various end uses also in Latvia. Unlike covered barley it is possible to use it in food products directly as inedible outer hull is only loosely connected to the kernel. New knowledge about hulless barley in Latvia was generated around the year 2000 when a collection was being gathered and different varieties were being tested in our conditions. In this time the intensive breeding work of hulless barley varieties also started. Two varieties Irbe and Kornelija are registered. By implementing several research projects the variety Kornelija was created and commercialization strategy was developed, expanding collaboration with grain processing and food production companies, plant breeders' rights are now granted to reach European and USA market. The aim of the study was to assess and compare the biochemical composition of variety Kornelija and that of other hulless barley varieties available on the European and North American market. The following HB varieties Kornelija (LV), Irbe (LV), Pihl (NO), Netto (NO), Gawrosz (PL), Naku (SW), SW Godiva (SW), CDC Hilose (CA), CDC Ascent (CA), CDC Fibar (CA), AF Lucius (CZ) were included in the field trials at AREI Stende Research Centre (57.1412° N, 22.5367° E) established from 2018 to 2020. Grain samples were analysed to parameters essential for functional food and dietary products such as crude protein/CP, total β-glucans/BG, crude fat/CF, resistant starch/RS, total dietary fibre/TDF, the total sugars content. For five selected HB varieties the composition of amino acids/AA and the range of dietary minerals (Zn, Mg, Cu, Fe, P, Ca) were assessed. Chemical composition varied ($p<0.05$) among all the varieties. The highest protein content formed variety Kornelija (196.6 g kg-1) and low amylose starch or waxy barley CDC Fibar (18.98%). BG among varieties varied from 42.8 to 57.9 g kg-1 with the highest ranking values for high amylose barley CDC Hilose and waxy barley CDC Ascent, following by varieties Kornelija (56.0 g kg-1) and Gawrosz (52.1 g kg-1). RS for the most tested HB varieties varied from 0.4 to 2.6%, only variety CDC Hilose had 10.6% of RS and 31.4% of total dietary fibre thus the uniqueness of this genotype was confirmed also in Latvian conditions. CF for genotypes with modified starch CDC Ascent and CDC Hilose was 3.0 and 3.3%, respectively. Variety Kornelija had the highest sum of essential AA showing comparatively higher proportion of Met, Cys, Hys and Phe, and significantly higher Mg (121-174 mg 100 g-1), Fe (49.5-73.6 mg kg-1) and Zn (30.8-38.1 mg kg-1) among tested HB samples. Overall, the HB variety Kornelija provided grains of excellent biochemical quality and together with high and low amylose barley genotypes they have a great potential as a raw material for their further use by food industry.

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KEYWORDS: whole barley grain, crude protein, β-glucans, resistant starch, essential amino acids

KNOCKOUT MUTATION OF PROTEASE INHIBITORS USING CRISPR/CAS9 FOR BETTER PROTEIN DIGESTIBILITY OF BARLEY GRAIN AS FEED

Michael Panting
Aarhus University, Dept. of Agroecology – Crop Genetics and Biotechnology, Research Center Flakkebjerg, Denmark

mpanting@agro.au.dk

Co-Authors:

Inger Bæksted Holme, Henrik Brinch-Pedersen
Aarhus University, Dept. of Agroecology – Crop Genetics and Biotechnology, Research Center Flakkebjerg, Denmark

Barley is an important crop mainly grown for feedstuff to livestock. However, the barley grain contains a high level of protease inhibitors interacting with proteases needed for proteolysis of feed proteins. The protease inhibitors provides a significant anti-nutritional effect when the grain is used as food or feed. In the current study we have edited endogenous protease inhibitors found in the barley grain. By removing these inhibitors, proteases in the food and feed matrix can act more efficient and provide amino acids to be taken up in the digestive tract. This will increase the value of the feed and decrease the secretion of non-digested protein into the environment. By using CRISPR/Cas, six different protease inhibitors expressed in the seeds have been knocked out. Some of the knock out mutants shows significantly less inhibition of selected proteases and significantly better proteolysis of barley grain storage proteins.

KEYWORDS: protease inhibitors, anti-nutritional factors, CRISPR/Cas, new breeding techniques

PASTING PROPERTIES OF BARLEY AND MALT AS A TOOL FOR BARLEY SELECTION VARIETIES WITH GOOD MALT QUALITY

Blanca Gómez-Guerrero
Latitud - LATU Foundation
bgomez@latitud.org.uy

Co-Authors:

Romina Lemus
Latitud-LATU Foundation

Tania De León , Valeria Lanaro
LATU - Technological Laboratory of Uruguay

Malsters require barley of known variety with consistent specific quality. Breeder's challenge is to develop new varieties that satisfy farmers (good yield) and market malting quality requirements. As starch is the major component of the barley grain, changes in its biophysical and biochemical properties allows have direct effects on its malting properties. Instrumental techniques currently used to determine these properties include differential scanning calorimetry (DSC) and rapid visco-analyzer (RVA). DSC determines thermal characteristics, gelatinization transition temperature and enthalpy of gelatinization. RVA was developed to measure the pasting properties of the starch and has several advantages including the small sample size, fast determination, and ability to set temperature profiles. RVA has been used mainly for barley sprouting determination. The aim of this study was to establish the rheological response of barley and malt varieties using a RVA instrument and its relationship with gelatinization temperature and malt quality parameters. This information will offer breeders a tool for screening of new malting barley varieties. Eleven elite barley varieties planted in one location in Uruguay were used. The barley samples were micromalted and malt quality parameters were determined according to methods based on the EBC standard. The viscosity profile of barley and malt was determined using RVA. The temperature and enthalpy of gelatinization of each sample was also determined by DSC. In barley and malt, the results showed significant differences ($p<0.05$) between varieties for RVA and DSC parameters. In barley, correlation analysis of these parameter and malt quality indicated a significant and positive relation between peak viscosity and fine extract (0.64), one of the most important parameters to determine the performance of malt in brewing industry. Malt results showed significant correlations between RVA and DSC parameters, mainly between gelatinization temperature and pasting temperature (0.83), allowing to predict the gelatinization temperature avoiding the use of DSC for all the samples. Correlation studies with malt quality parameters indicated a strong correlation between several of the RVA parameters and malt quality parameters, among which the most relevant are peak viscosity with micromalting quality index (-0.84) and peak viscosity with beta glucans (0.86). From these results it can be concluded that the RVA is a good tool to screen malting barley cultivars in early generations selecting based on malt quality. With one determination, malt quality behavior can be predicted, in barley or malt grain, and deciding if continue or discard materials is essential for breeders. By using different temperature curves, the qualities and composition of the cultivars can be known in greater depth. Further studies are planned to be conducted in different locations and harvest years, to include the environment as a variable.

Aknownledgements: We want to thank to Uruguayan barley board members (malting companies MUSA and MUSA; malting barley breeding programs INIA and FAGRO; and INASE) to allow us to use the barley samples.

KEYWORDS: malt quality, barley selection, RVA, DSC, breeding tool

GENETIC AND DATABASE RESOURCES: HARNESSING DIVERSITY

AT THE DARK SIDE OF BARLEY DIVERSITY – THE GAP BETWEEN DISCOVERY AND USER-FRIENDLY ACCESS

Nils Stein
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Seeland, Germany
stein@ipk-gatersleben.de

The genomic revolution has provided the barley research community with almost unlimited access to molecular genetic diversity information of the species. Initially, genetic maps were populated by different types of EST-based molecular marker types. Soon after, microarray- and next generation sequencing (NGS)-based transcriptome analysis provided new tools to barley functional genomics. NGS ultimately allowed to gain access to the entire barley genome, more recently even the pan-genome of the crop species including also non-domesticated barley, and in the near future even of all of barley wild relatives. This development introduced a new challenge to the field and specifically to the individual research units: basically, all molecular genomic methods yield large raw datasets and typically require powerful compute infrastructures for efficient analysis.
Project-specific databases have made datasets often only partially and sometimes only temporarily accessible. Sustainable access and the possibility of extensive reuse of data is a challenge that is not specific to the barley research area, however, also in barley research there is a pressing need for the systematic introduction at broad-scale of FAIR data principles in order to facilitate future integrated analysis of large genomic and phenotypic datasets over time. The presentation will introduce the problem, spot-light the current status and will make a proposal for foreseeable developments that need stronger community awareness and input.

KEYWORDS: FAIR data, database

THE DOUBLE ROUND ROBIN POPULATION UNRAVELS THE GENETIC ARCHITECTURE OF GRAIN SIZE IN BARLEY

Asis Shrestha
Institute for Quantitative Genetics and Genomics of Plants, Heinrich Heine University, Dusseldorf
shrestha@hhu.de

Co-Authors:

Francesco Cosenza, Po-Ya Wu, Delphine van Inghelandt, Federico A. Casale, Marius Weisweiler, Benjamin Stich
Institute for Quantitative Genetics and Genomics of Plants, Heinrich Heine University, Dusseldorf

Jinquan Li
Max Planck Institute for Plant Breeding Research, Cologne

Grain number, size and weight primarily determine the yield of barley. Although the genes regulating grain number are well studied in barley, the genetic loci and the causal gene for sink capacity are poorly understood. Therefore, the primary objective of our work was to dissect the genetic architecture of grain size and weight in barley. We used a multi-parent population developed from a genetic cross between 23 diverse barley inbreds in a double round robin design. Seed size-related parameters such as grain length, grain width, grain area and thousand-grain weight were evaluated in the HvDRR population comprising 45 recombinant inbred line sub-populations. We found significant genotypic variation for all traits and observed a heritability of 84 % or higher across four environments.
The results of the QTL detection indicate that the genetic architecture of grain size is more complex than reported previously. In addition, both cultivars and landraces contributed positive alleles at grain size QTLs. Candidate genes identified using genome-wide variant calling data for all parental inbred lines indicated overlapping and potential novel regulators of grain size in cereals. Furthermore, our results indicated that sink capacity was the primary determinant of grain weight in barley.

KEYWORDS: Barley, grain size, grain weight, multi-parent population, QTL

CGIAR BARLEY BREEDING TOOLBOX: A DIVERSITY PANEL TO FACILITATE BREEDING AND GENOMIC RESEARCH IN THE DEVELOPING WORLD

Outmane Bouhlal
Biodiversity and Crop Improvement Program (BICP), International Centre for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco. // Team of Anthropogenetics and Biotechnologies, Faculty of Sciences, Chouaib Doukkali University, El-Jadida, Morocco

outmanebouhlal1994@gmail.com

Co-Authors:

Andrea Visioni, Miguel Sanchez-Garcia
Biodiversity and Crop Improvement Program (BICP), International Centre for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco

Ramesh Pal Singh Verma
Indian Institute of Wheat and Barley Research, Karnal, India

Mostafa Kandil
Team of Anthropogenetics and Biotechnologies, Faculty of Sciences, Chouaib Doukkali University, El-Jadida, Morocco

Sanjaya Gyawali
Sakata Seed America Inc., 16943 Dike Rd, Mount Vernon, WA 98273

Flavio Capettini
Field Crop Development Centre, Alberta Agriculture and Forestry, 5030 50th Street, Lacombe, AB T4L1W8, Canada

The efficient use of the new genotyping technologies and genomic research for breeding are important to achieve solid genetic gains in a global scenario of climate instability. However, many breeding programs, mostly in developing countries, still cannot afford these technologies. For it, we aim to select and assemble a new association mapping panel, representative of the germplasm grown in the Developing World; covering a wide range of genetic variability, morphophysiological relevant traits and phenology groups and of public domain to facilitate access to breeders and researchers in the Worldwide.
With the mandate to develop new barley genotypes and cutting-edge research for the developing World, the CGIAR Global Barley Breeding program (ICARDA) assembled and genotyped a Global Barley Panel (GBP) of 530 genotypes, 350 of them of CGIAR origin or cultivated landraces. This panel represents a wide range of row types, end-uses, growth habits and large geographical and environmental distribution. The GBP accessions were genotyped using the barley Infinium iSelect 50K chip, then used to evaluate the genetic relationships and diversity of the panel. Four SPN markers associated with phenology loci together with their allelic combinations (AC) were used to assess the extent of genetic diversity based on phenology. A total of 40,342 SNP markers were found polymorphic in the GBP and displayed an average PIC of 0.35. The principal component analysis (PCA), neighbor-joining clustering and population structure analysis identified 8 subpopulations, mainly differentiated by row type and geographical origin. AMOVA analysis showed that row type and origin accounted for 10.6% and 12.3% of genetic diversity, respectively. Six-row barleys contributed to a higher share of genetic variation and were more diverse as compared to two-row genotypes. Among origins, CGIAR material showed the largest diversity and the highest number of polymorphic loci (99.76% of all polymorphic SNPs in the GBP are present in CGIAR entries). The SNP associated with HvCEN explained 11.1 % of the genetic variation while the 16 ACs explained 11.44%.

Using the genotypic data, we assembled a subset of the GBP, named CGIAR Barley Breeding Toolbox (CBBT), made of 250 accessions of CGIAR or open access origin capturing the majority of the GBP's allelic diversity, to serve as a publicly available panel. The selection was done using Mean of Transformed Kinships method. The CBBT showed a good coverage of most of the PCA spectra except the most negative values in PC1, where most European, Canadian and USA varieties are located. All the subpopulations identified in the GBP are represented in the CBBT as well as all the phenological ACs.
The CBBT entries together with their genotypic data will be made available to breeders and researchers worldwide to serve as a collaborative tool to underpin the genetic mechanisms of traits of interest for barley cultivation, especially for the developing World.

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KEYWORDS: Global Barley Panel, genetic diversity, population structure, phenology groups, barley breeding toolbox

COMPREHENSIVE EXPRESSION ATLAS OF SIX TISSUES COUPLED WITH HIGH DENSITY SNP DATA PROVIDES A RESOURCE FOR STUDIES AND CHARACTERIZATION OF EUROPEAN TWO-ROWED SPRING BARLEYS AND IDENTIFIED A MAJOR HAPLOTYPE SWITCH ON CHROMOSOME 5H

Ronja Wonneberger
Cell and Molecular Sciences, James Hutton Institute, Invergowrie, Dundee, DD2 5DA, Scotland
ronja.wonneberger@hutton.ac.uk

Co-Authors:

Miriam Schreiber, Max Coulter,
Division of Plant Sciences, University of Dundee, James Hutton Institute, Invergowrie, Dundee, DD2 5DA, Scotland

Allison Haaning, Gary Muehlbauer
Department of Agronomy and Plant Genetics, University of Minnesota, 1991 Upper Buford Circle, St. Paul, MN 55108, USA

Robbie Waugh
Cell and Molecular Sciences, James Hutton Institute, Invergowrie, Dundee, DD2 5DA, Scotland. Division of Plant Sciences, University of Dundee, James Hutton Institute, Invergowrie, Dundee, DD2 5DA, Scotland. School of Agriculture and Wine & Waite Research Institute, University of Adelaide, Waite Campus, Glen Osmond, SA, 5064, Australia

Nils Stein
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), OT Gatersleben, Corrensstrasse 3, D-06466 Stadt Seeland, Germany. Center for Integrated Breeding Research (CiBreed), Georg-August-University, Göttingen, Germany

Improving varieties requires a thorough understanding of the genetic architecture of morphological and physiological traits, as well as of the interaction and regulation of genes affecting these traits. In the ERA-CAPS-funded 'BARN' project, we generated several resources to facilitate studies of the European two-rowed spring barley germplasm over the past 100 years. Using the new reference transcriptome BaRTv2, we developed an expression atlas of six tissues and developmental stages from seedling to grain filling in a panel of 211 two-rowed spring barley cultivars representing the European breeding germplasm of the last century, complemented with a high-density SNP dataset of almost 500,000 SNPs derived from RNAseq of these tissues and low-coverage WGS sequencing. The panel was phenotyped for more than 20 traits, mainly earliness, grain and spike traits, in field trials in Gatersleben (Germany), Dundee (Scotland) and St. Paul, Minnesota (USA) in 2019 and 2020. Genome-wide (GWAS) and transcriptome-wide association studies (TWAS) of these important traits are currently underway.
We identified a region between 70 and 320 Mbp on chromosome 5H containing several genes whose expression was associated with height and grain traits in all tissues. Notably, a subset of ~30 genes showed no or low expression in a subset of older cultivars released before the 1990s but was highly expressed in newer cultivars released after the 1980s. FST values over 0.8 indicate that this region is almost completely fixed for different alleles in the two groups. This region was also highly significant in an EigenGWAS using the first principal component as the phenotype, indicating that this region is a major determinant of population differentiation. Only one of the 58 lines released after 2000 possessed the old haplotype, indicating that there has been a strong direct or indirect selection pressure on the new haplotype. The region is further characterized by flanking structural rearrangements between Golden Promise (older cluster) and Barke (newer cluster) at 70 and 320 Mbp.

The oldest line in the panel carrying the newer haplotype is Union, released in the 1950s and derived from Southern German lines and landraces. A screen of the two-rowed spring barley collection of the Federal ex-situ gene bank collection at IPK Gatersleben in Germany showed that among the multiple entries for the progenitors of Union, most carry the old haplotype. However, a few accessions carry the new haplotype. It is therefore not clear when and where the new haplotype was initially introduced into the modern European germplasm, but our analysis suggests southeastern European landraces as a potential origin.

KEYWORDS: genomics, transcriptomics, population genetics, multi-omics

QUEST FOR AN ELUSIVE DEVELOPMENTAL GENE IN BARLEY

Agatha Walla
Institute of Plant Genetics, Heinrich-Heine-Universität Düsseldorf
walla@hhu.de

Co-Author:

Maria von Korff
Institute of Plant Genetics, Heinrich-Heine-Universität Düsseldorf

Recent advances in barley genomics and accompanying cost reductions in NGS technologies made it feasible to exploit mutant collections and increased the number of identified candidate genes for investigated traits. These traits are often of high agronomical value, such as the number of seeds, the number of spikes and tillers or plant height which together form the overall shoot architecture. Hence, identification of underlying genes fosters our understanding of the genetic regulation of shoot architecture development and unfolds new opportunities in shaping high yielding barley ideotypes.
Here, we describe the twists and turns in the process of identifying a gene using NGS technologies in a forward genetics approach. We investigated a pleiotropic high-tillering mutant producing short culm nodes leading to a dwarf plant architecture. In contrast to other high-tillering mutants classified as many noded dwarf, this mutant developed just one additional node at the main stem compared to wild type. However, negative correlations between biomass and yield were observed in this mutant as reported previously for other many noded dwarf mutants. Previous attempts on identifying causal mutations of this phenotype using a mapping by RNA sequencing approach were unsuccessful. Only a combination of whole genome sequencing (WGS) and maker-assisted mapping in a biparental population allowed to identify a candidate mutation. This mutation comprised a large inversion on the long arm of chromosome 7H. Nevertheless, this structural mutation did not reveal a causative gene. Allelism tests with other high-tillering mutants identified allelic lines which were further used to characterize the mutant. Interestingly, the F1 generation of a cross with a known tillering gene on chromosome 7H showed incomplete complementation of the phenotype, although the investigated mutant showed a much milder phenotype compared to the known tillering mutant. However, resequencing of the tillering gene showed no mutations in the investigated mutant line. Only by combining WGS, RNAseq and complementation test data, we now can propose that the underlying mutation is located in the cis-regulatory region of the previously described tillering gene with strong effects on its expression. We hypothesize that the cis-regulatory variation and resulting expression changes of the tillering gene can modulate the phenotypic expression allowing a weaker consequence on shoot architecture traits.

KEYWORDS: gene identification, barley mutant, shoot architecture, development

A REFERENCE-GUIDED TILLING BY AMPLICON-SEQ PLATFORM SUPPORTS FORWARD AND REVERSE GENETICS IN BARLEY

Congcong Jiang
Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China
jiangcongcong@caas.cn

Co-Authors:

Miaomiao Lei, Lijie Shi, Yu Cai, Zongyun Feng
College of Agronomy, Sichuan Agricultural University, Chengdu, China

Yu Guo, Axel Himmelbach
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Seeland, Germany

Guangqi Gao, Yanlong Jin, Shenghui Zhou, Qiang He, Jinhong Kan, Fengying Duan, Lihui Li, Jun Liu, Jing Zhang, Ping Yang
Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China

Xuefeng Yao, Chunming Liu
Institute of Botany, Chinese Academy of Sciences, Beijing, China

Georg Haberer, Manuel Spannagl
Plant Genome and Systems Biology (PGSB), Helmholtz Center Munich, German Research Center for Environmental Health, Neuherberg, Germany

Nils Stein, Martin Mascher
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Seeland, Germany

Barley is a diploid species with a genome smaller than those of other members of the Triticeae tribe, making it an attractive model for genetic studies in Triticeae crops. The recent development of barley genomics has created a need for a high-throughput platform to identify genetically uniform mutants for genes functional investigation. In this study, we reported an ethyl methanesulfonate (EMS)-mutagenized population consisting of 8,525 M3 lines in the barley landrace 'Hatiexi' (HTX), which we complement with a high-quality de novo assembly of a reference genome for this genotype. The mutation rate within the population ranged from 1.51 to 4.09 mutations per Mb, depending on the treatment dosage of EMS and the mutation discrimination platform employed for genotype analysis. We implemented a three-dimensional DNA pooling strategy combined with multiplexed amplicon-sequencing to create a highly efficient and cost-effective TILLING (Targeting Induced Locus Lesion in Genomes) platform in barley. Mutations were successfully identified from 72 mixed amplicons within a DNA pool containing 64 individual mutants, and from 56 mixed amplicons within a pool containing 144 individuals. We discovered abundant allelic mutants for dozens of genes, including the barley green revolution contributor gene Brassinosteroid insensitive 1 (BRI1). As a proof of concept, we rapidly determined the causal gene responsible for a chlorotic mutant by following the MutMap strategy, demonstrating the value of this resource to support forward and reverse genetic studies in barley.

KEYWORDS: Barley, Mutagenesis, TILLING, Amplicon-seq, Genetics

BREEDING FOR HETEROGENEOUS SPRING BARLEY POPULATIONS: FIRST RESULTS ON SOME IMPROVEMENT METHODS

Linda Legzdiņa
Institute of Agricultural Resources and Economics, LATVIA
linda.legzdina@arei.lv

Co-Authors:

Māra Bleidere, Dace Piliksere
Institute of Agricultural Resources and Economics, LATVIA

Heterogeneous populations can provide large diversity within a single crop field, are able to evolve within the time and adopt to a specific cultivation environments. The research history on evolutionary barley populations starts back in the beginning of 20th century. Nowadays heterogeneous material become topical due to rapid development of organic agriculture and its advantages especially under unfavourable and stress environments.

Our research aims for development and further improvement of composite cross populations (CCPs). We did test some methods targeted to reduce the workload needed for CCP development and to improve existing populations: (1) application of male sterility in crossing, (2) selection for loose smut resistance by molecular marker within a population, (3) selection for best-performing lines within a population and combining them in a mixture, (4) crossing of existing CCP to additional parents to improve some disadvantageous traits, and (5) natural selection under artificial infection and/or provocative background to several diseases. Testing was done in 2021.

Two populations were created using the same set of parents: (a) by using dialell crosses between 11 genotypes and (b) by pollination of eight male sterile lines with 11 genotypes each. Comparing both F3 populations resulted in on average 11% lower yield for ms CCP. It had also slightly lower early vigour and weed suppressive ability, higher infection with powdery mildew, higher protein content and N use efficiency (NUE).

Selection for Un8 loose smut resistance was done in one hulled and one hulless CCPs of the same origin. Resistant plants were bulked, multiplied and compared to the unselected populations. The amount of resistance was higher in hulless than hulled population (37 vs 19%). For covered Un8 CCP on average 10% yield reduction was found, whereas hulless Un8 CCP provided significant yield increase by 13% in one of two sites. Un8 CCPs had lower powdery mildew resistance.

The yield of selected line mixtures did not significantly differ from the four respective CCPs, however, mixture selected from CCP created by male sterile crosses surpassed the unselected population by 17% and was superior also for early vigour and weed suppressive ability. There was a trend for line mixtures to surpass CCPs for tillering ability but CCPs did better according to severity of leaf diseases.

One CCP was crossed to three advanced pure lines to improve resistance to loose smut, yield and NUE. The newly created population (F3) did not significantly differ for yield, but had advantages for early vigour and traits related to weed suppressive ability, mildew severity, and protein in comparison to the original F9 population.

Natural selection in six CCPs grown for three seasons under artificial infection/provocative background provided some advantage in respect to net blotch, however, no general positive gain for smuts, powdery mildew and Fusarium head blight was noticed.

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KEYWORDS: composite cross populations, male sterility, disease resistance, line mixtures, selection

CHARACTERISATION OF A DIVERSE PANEL OF BARLEY VARIETIES UNDER IRISH ORGANIC CONDITIONS TO IDENTIFY KEY TRAITS FOR MULTI END-USES

Laura Paire
University College Dublin (UCD, Ireland), Irish Research Council (postgraduate scholarship)
laura.paire@ucdconnect.ie

Co-Authors:

Tomás McCabe
University College Dublin (UCD, Ireland)

This project aims to identify sources of genes to build a sustainable spring barley crop for organic farming systems. In barley production in a maritime climate, grain yield and quality show higher variability across years and locations in a minimal input regime compared to high inputs. Therefore, plant breeding programs should consider the specific needs of organic cereal farming by selecting locally adapted varieties for small-scale production at low input levels and prioritise traits such as disease resistance or straw strength. Barley is currently primarily used for feed and malting and very little for food, despite its high grain nutritional properties (low fat, high protein, high beta-glucan). However, unlike the commonly grown "hulled" barley varieties that require a process to make grains edible, "naked" barley lines produce whole grains with more food product applications and higher added value to the farmer with improved financial security by providing multiple market outlets.

A diverse panel of 247 spring naked barley accessions was grown for three years (2019, 2020, 2021) on an organic certified field located in Camolin (Co.Wexford, Ireland), following a type II modified augmented design with 3 check lines (Full Pint, CDC Clear, and Annapurna). The panel comprises 60 lines from Genebanks (GRIN, RIB), with seeds collected across the globe and dating back up to the 1940s. The remaining lines are from recent US breeding programs (OSU, WSU). Field observations were carried out to record growing degree days to booting, heading, anthesis, and physiological maturity; plant height; diseases scores; straw strength. On harvested grain samples, data was collected on yield, 1000-kernel weight, threshing ability, protein level, and beta-glucan content. Genotyping data on 44,040 single nucleotide polymorphism (SNP) markers was obtained with a 50K SNP Illumina chip.

A set of 6,505 independent markers was used to characterise the genetic diversity across the panel. Principal component analysis showed mild genetic population structure, while the genetic relatedness effect is stronger. Hierarchical clustering analysis identified six groups of lines, mainly differentiated by their breeding history. Major diseases observed on barley in Ireland were present in the study, and powdery mildew and brown rust were the most prevalent across the panel. Variation between years was observed due to contrasting weather conditions. In 2021, warmer and dryer weather over the summer period led to lower disease pressure compared to 2020. Average yield increased by 50% every year (from 80 to 182 g/plot), and mean 1000-kernel weight nearly doubled between 2019 and 2021 (26-41g). Overall, agronomic data show significant variation among genotypes for most traits, indicating good opportunities for selection within the panel. Moreover, broad-sense heritability values are high (>70%), suggesting the possibility to identify marker-trait associations.

KEYWORDS: naked barley, organic farming, diversity panel, plant breeding, single nucleotide polymorphism

RAPID IDENTIFICATION OF CAUSAL MUTATIONS BY WHOLE GENOME SEQUENCING OF F2 PHENOTYPIC BULKS

David Stuart
Lund University
david.stuart@biol.lu.se

Co-Authors:

Shakhira Zakhrabekova, Mats Hansson
Lund University

Barley research has a deep history of utilizing genetic variation thanks to large germplasms around the world that arose during domestication and the induction of mutations by early geneticists and later by plant breeders. This history predates the 1953 publication by Watson and Crick on the structure of DNA – a time when identifying the underlying genetic variation resulting in these fascinating phenotypes was not possible. As the world moved into the molecular era and then the genomics era, small genome species such as rice and Arabidopsis had a clear advantage. The barley renaissance is now. Here we present a simple and cost effective methodology for identification of mutations from F2 mapping populations in barley that is also efficient in recombination poor chromosomal locations. In short, plant material is collected from approximately 200 F2 individual showing the phenotype.
The material is pooled and genomic DNA is isolated and whole genome sequencing is performed. Since SNPs linked to the causal mutation will be homozygous and those that are unlinked will be heterozygous, the chromosomal location of the mutation can be localized by plotting allele frequencies along each chromosome. For mutations located in recombination poor centromeric regions this may yield an interval representing a majority of the chromosome while for telomeric regions recombination may reduce the mapping interval to only 100 million base pairs. Variant calling allows direct identification of candidate mutations in the mapped interval. Even for centromeric regions this generally yields only a few and in some cases just a single candidate gene.
We have successfully identified causal SNPs, small deletions, as well as large deletions that remove whole chromosomal segments. In this way, we have recently identified six, and counting, genes deficient in barley chlorophyll mutants and thanks to the availability of allelic mutants we have been able to verify that the correct mutation was indeed identified.

KEYWORDS: mutant, gene idenitification, NGS sequencing

A REFERENCE-GUIDED TILLING BY AMPLICON-SEQ PLATFORM SUPPORTS FORWARD AND REVERSE GENETICS IN BARLEY

Ping Yang
Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China
yangping@caas.cn

Co-Authors:

Miaomiao Lei, Lijie Shi, Zongyun Feng
College of Agronomy, Sichuan Agricultural University, Chengdu, China

Congcong Jiang, Guangqi Gao, Yanlong Jin, Yu Cai, Shenghui Zhou, Qiang He, Jinhong Kan, Fengying Duan, Lihui Li, Jun Liu, Jing Zhang
Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China

Xuefeng Yao, Chunming Liu
Institute of Botany, Chinese Academy of Sciences, Beijing, China

Georg Haberer, Manuel Spannagl
Plant Genome and Systems Biology (PGSB), Helmholtz Center Munich, German Research Center for Environmental Health, Neuherberg, Germany

Yu Guo, Axel Himmelbach, Nils Stein, Martin Mascher
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Seeland, Germany

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In this study, we reported an ethyl methanesulfonate (EMS)-mutagenized population consisting of 8,525 M3 lines in the barley landrace 'Hatiexi' (HTX), which we complement with a high-quality de novo assembly of a reference genome for this genotype. The mutation rate within the population ranged from 1.51 to 4.09 mutations per Mb, depending on the treatment dosage of EMS and the mutation discrimination platform employed for genotype analysis.
We implemented a three-dimensional DNA pooling strategy combined with multiplexed amplicon-sequencing to create a highly efficient and cost-effective TILLING (Targeting Induced Locus Lesion in Genomes) platform in barley. Mutations were successfully identified from 72 mixed amplicons within a DNA pool containing 64 individual mutants, and from 56 mixed amplicons within a pool containing 144 individuals. We discovered abundant allelic mutants for dozens of genes, including the barley green revolution contributor gene Brassinosteroid insensitive 1 (BRI1).
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KEYWORDS: Barley, Mutagenesis, TILLING, Amplicon-seq, Genetics

BARPLEX V1.0: A MULTIPLEX PCR-BASED ENRICHMENT OF GENOME-WIDE SHORT SEGMENTS THAT ENABLE GENETIC STUDIES IN BARLEY

Ping Yang
Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China
yangping@caas.cn

Co-Authors:

Guangqi Gao, Yu Cai, Congcong Jiang, Qiang He, Sarah Tasnim, Jun Liu, Jing Zhang
Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China

Luxi Yan, Zongyun Feng
College of Agronomy, Sichuan Agricultural University, Chengdu, China

Over the past 15 years sequencing methodologies have advanced greatly enabling high-throughput sequencing based genotyping of crop plants. In this study, we developed BarPlex v1.0, a robust and cost-efficient genotyping approach in barley (Hordeum vulgare L.). In this multiplex PCR-based amplification of five-hundred genome-wide segments, followed by high-throughput sequencing of barcoded PCR products, we obtained hundreds to thousands of polymorphic markers. Comparison of genotyping with BarPlex v1.0 to genotyping-by-sequencing (GBS) revealed a similar genetic diversity.
The polymorphic markers revealed by BarPlex v1.0 were highly accurate, with an average sequencing depth >700x and a data missing rate <0.5%. By analyzing 1,068 genotypes of wild barley (Hordeum vulgare ssp. spontaneum L.), Tibetan semi-wild barley (Hordeum agriocrithon; brittle rachis), landraces, cultivars, as well as an F2 population, this assay has been robust in studies of population diversity, variety pedigree, heterozygosity discrimination, linkage mapping, as well as genome-wide association study (GWAS).
Notably, a diversity analysis in a population of Tibetan semi-wild barley suggested a close relationship with Chinese landraces, but a dramatic decrease in its genetic diversity, inferring that Tibet was unlikely a center of domestication for the native wild barley.

KEYWORDS: Barley, Tibetan semi-wild barley, H. agriocrithon, Genotyping, Multiplexed enrichment

QTL ANALYSIS OF ROOT TRAITS IN SEEDLINGS OF A BARLEY RIL POPULATION

Alejandra Cabeza
EEAD-CSIC
acabeza@eead.csic.es

Co-Authors:

Ana M. Casas, Ernesto Igartua
EEAD-CSIC

Root systems have not been thoroughly explored in breeding programs. Plant breeding will benefit from the study of root diversity. Studying root system architecture (RSA) will lead to a better understanding of the patterns of root development in relation with soil exploration for the acquisition of water and nutrients. The aim of this study is to evaluate root system diversity in a population of barley (Hordeum vulgare L.) recombinant inbred lines (RILs), and to identify quantitative trait loci (QTLs) for root traits potentially useful in breeding programs.
A population of 114 RILs from the Orria x Plaisant cross, an elite Spanish breeding population, was tested for RSA traits under controlled and repeatable conditions. The RILs were genotyped with barley OPA1. The lines were evaluated at seedling stage, using a rhizoslide system, which is low-cost, medium-throughput method, amenable to breeding operations. A sandwich composed of a PVC plate, black cardboard sheet, filter paper, and a plastic sheet, A-4 size, with the lower end submerged in a container with distilled water, was used to grow the seeds.
The population was previously characterized for germination speed, to plan sowings. Six pre-germinated seedlings for each RIL were grown, one per sandwich, in a growth chamber for 7 days at 22/18˚C and 12/12 h photoperiod. After that, roots were scanned using a flatbed scanner, at 330 ppi, and analyzed using the software RootNav. The set of morphological and quantitative traits were then subjected to QTL analysis. QTL for total root length, root number, root angle and other traits were found and will be reported. A field trial with the 10% families showing extreme values for root angle, to validate the results in the field, is ongoing.

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KEYWORDS: barley, root, seedling, linkage mapping, QTL

AGROCLIMATIC DISTRIBUTION OF BARLEY LANDRACES OVER THE MEDITERRANEAN BASIN

Ernesto Igartua
EEAD-CSIC
igartua@eead.csic.es

Co-Authors:

Francesc Montardit-Tarda, Ana M. Casas, Bruno Contreras-Moreira
EEAD-CSIC

Alessandro Tondelli, Luigi Cattivelli, Agostino Fricano
CREA

Hakan Ozkan
University of Çukurova

Andrea Visioni
ICARDA

A collection of circa 1000 barley landraces from the Mediterranean basin has been gathered and multiplied by SSD. This collection, the Mediterranean barley reference set, is the basis for genomics and physiological approaches to study barley adaptation in response to climatic factors. To explore the extent and distribution of genetic diversity it was genotyped with SNP markers using genotyping-by sequencing. 382,605 markers were obtained. For further analyses, 66,047 and 42,388 were kept after filtering for minor allele frequency at or above 1% and sample depth of 10% or 30%, respectively.
This report presents the population structure revealed after the analysis of the genetic diversity of this set, enriched with another 1779 accessions of the Mediterranean and neighboring regions, provided by IPK Bridge portal (König et al., 2020), published in Milner et al (2019). The combination of both datasets, with a read depth of 5, resulted in 10,754 common SNPs providing good genome coverage.
Population structure was analyzed with software sNMF (Frichot et al., 2014). Twelve groups were distinguished. Spike type was one of the main drivers of genetic differentiation, but other major factors must have been present. Some groups had a wide geographic distribution, whereas others were characteristic of smaller regions, suggesting a layered dynamic of landrace spread over the region, and specific adaptations to local conditions. The relationship of the genetic diversity with climatic factors is analyzed through several statistical procedures, using climatic variables extracted from the WorldClim 2 database, adjusted for estimated sowing and harvesting dates from the SAGE database (Sacks et al., 2010).

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KEYWORDS: barley, ecogeography, genetic diversity

GWAS META-ANALYSIS FOR AGRONOMIC TRAITS IN TWO-ROWED SPRING BARLEY

Francesc Montardit-Tarda
EEAD-CSIC
fmontardit@eead.csic.es

Co-Authors:

Ernesto Igartua, Ana M. Casas
EEAD-CSIC

Alessandro Tondelli, Agostino Fricano, Luigi Cattivelli
Centre for Genomics and Bioinformatics, CREA

William T.B. Thomas, Joanne Russell, Hazel Bull, Luke Ramsay, Robbie Waugh
James Hutton Institute

Andrea Visioni
ICARDA

Salar Shaaf, Gianluca Bretani, Laura Rossini
Universita de Milano

Klaus Pillen, Andreas Maurer
Martin Luther University Halle-Wittenberg

Rajiv Sharma, Andrew J Flavell
University of Dundee at JHI

Benjamin Kilian
Crop Trust, Bonn

Alan Schulman
Natural Resources Institute Finland (LUKE), Finland

Two-rowed spring varieties constitute one of the main focus of barley breeding efforts in Europe. Two EU projects, ExBarDiv and ClimBar, analyzed the agronomic responses of diverse barley panels in field trial networks, with locations in Finland, Germany, United Kingdom, Italy, Morocco, and Spain, during the 2009, 2010 (ExBarDiv), 2016 and 2017 (ClimBar) harvest seasons. After data curation sixteen field trials, and a subset of 151 spring two-rowed accessions were kept for further analysis. Best linear unbiased predictors (BLUPs) for flowering time, plant height, thousand kernel weight, and grain yield, per accession and trial were calculated. Accessions were genotyped with the 50k Illumina Infinium SNP Array. After imputation, markers were filtered (MAF>0.05). Genome-wide association (GWAS) was performed for each trial, using the mixed linear model (MLM) implemented in the GAPIT R package, with a genomic kinship matrix for adjustment of relatedness. The results of single-environment analyses were meta-analysed with the software METAL. The significance threshold for meta-association was chosen as the minimum -log10 (p-value) found after running meta-analyses with 1000 permutations of the p-values of each trial. Confidence intervals for each peak were identified as the points were local LD decay fell below background LD, or, when that fit was not good, by chromosomic LD decay. Single environment analyses found few remarkable associations. However, meta-analyses identified many potential peaks, thanks to the extra robustness provided by the joint analysis of a large number of trials. A second meta-analysis guided by the main genotype-by-environment interaction patterns manifested per trait, pointed at the presence of some QTLs specific to latitude (plant height) and sowing date (flowering time). Each QTL region was then enriched with exome capture marker data, and meta-analysed following the same procedure. In several peaks, exome capture data were more associated than the flag-marker. Some of these markers indicate putative candidate genes, due to homology with Arabidopsis thaliana or other species. The best candidate genes for each trait will be discussed.

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KEYWORDS: GWAS, spring barley, yield, flowering, thousand kernel weight

ENVIRONMENTAL ASSOCIATION MAPPING AS AN APPROACH TO CHARACTERIZE AND UTILIZE EXOTIC BARLEY GERMPLASM

Che-Wei Changa
University of Hohenheim, Stuttgart, Germany
changc@uni-hohenheim.de

Co-Authors:

Eyal Fridman
Plant Sciences Institute, Agricultural Research Organization (ARO), The Volcani Center, Rishon LeZion, Israel

Martin Mascher, Axel Himmelbach
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Seeland OT Gatersleben, Germany

Susanna Boxberger, Claus Einfeldt
Ackermann Saatzucht GmbH, Irlbach, Germany

Johannes Wittman, Roland Geyer
lifespin GmbH, Regensburg, Germany

Antonio Aguinaga
Quilmes, Argentina

Gary Hanning, Nikki Else
Anheuser-Busch InBev, USA

Karl Schmid
University of Hohenheim, Stuttgart, Germany

Landraces and wild relatives are valuable genetic resources in barley breeding because adaptation to diverse environments provides favorable genetic variation, such as for improving climate resilience. To facilitate the utilization of genebank collections, one approach is to explore the association between genomic and environmental variation.
Based on landscape genomic analyses, we found evidence that wild barley from the Southern Levant adapted to water deficiency but observed a strong effect of neutral evolutionary processes on genetic variation (Chang et al. 2022). To utilize a similar landscape genomic approach with global cultivated barley landraces, knowledge of geographic origins (GO) is essential, but this information is usually missing. Thus, we implemented a neural network (NN) approach to recover the missing GO data of ~10,000 non-georeferenced landraces. By training NN using ~1,600 geo-referenced landraces with 558k GBS-derived SNPs, cross-validation revealed a prediction accuracy (R^2) of 0.99 and 0.94 for longitude and latitude, respectively. Since flowering time plays an important role in adaptation, we considered flowering time genes as benchmarks of adaptive loci to evaluate if the inclusion of non-georeferenced samples can improve the power of genome-environment association (GEA) scans.
By incorporating the inferred GOs of non-georeferenced landraces, GEA scan of temperature seasonality based on all landraces detected a strong signal (-log10(P)=194) at the ~209 kb upstream of CEN, which was not identified by the GEA scans using only geo-referenced accessions (n=~1,600). Although further validation is required, the finding may suggest the usefulness of GO inference in identifying adaptive loci. To develop strategies for utilizing differentially adapted exotic germplasm identified with landscape genomics, we are investigating the association between complex traits and genetic backgrounds.
For this, we estimated kernel metabolites (KMs) and malting quality (MQ) traits of 400 doubled haploid (DH) lines derived from F1 elite crosses and 400 DH lines derived from BC1 or BC2 backcross lines of elite

malting barley with landraces and wild barleys. KMs and MQs were evaluated in two-year trials at two locations in Argentina. We observed that KMs more positively correlate with MQ in the populations with elite parents than in the populations with landrace or wild barley parents. This suggests that KMs and MQs experienced artificial selection in the same direction. We are currently using GWAS and genomic prediction to investigate the genetic factors underlying observed differences and evaluate the potential of high-throughput metabolite measurement in breeding programs. In summary, leveraging environmental data, advanced computational approaches, and large-scale genotyping and phenotyping improves our knowledge of genebank collections to assist in decision-making and accelerating the development of climate-resilient varieties.

KEYWORDS: genebank, landscape genomics, geographical origin inference, metabolite, malting quality

GENOME WIDE ASSOCIATION MAPPING IDENTIFIES MAJOR QTLS AFFECTING SEMINAL ROOT TRAITS IN BARLEY

Giuseppe Sangiorgi
Department of Agricultural and Food Sciences (DISTAL), University of Bologna, viale Fanin 44, 40127, Bologna, Italy

giuseppe.sangiorgi2@unibo.it

Co-Authors:

Giuseppe Sciara, Serena Rosignoli, Roberto Tuberosa, Silvio Salvi
Department of Agricultural and Food Sciences (DISTAL), University of Bologna, viale Fanin 44, 40127, Bologna, Italy

Agostino Fricano, Alessandro Tondelli
CREA – Research Centre for Genomics and Bioinformatics, Via S. Protaso 302, 29017, Fiorenzuola d'Arda, Italy

A better knowledge about the genetic basis of root trait variation is crucial to successfully target drought tolerance or nutrient-use efficiency in breeding programs. In this study we investigated the genetic control of seminal root traits in a barley germplasm collection (WHEALBI) representative of the genetic variation in this species. Average root length (ARL), lateral root density (LRD), lateral root length (LRL), root dry weight (RDW), root growth angle (RGA), root total length (RTL), and seminal root number (SRN) were collected using a semi-hydroponic system at 13 days after germination.
All traits showed a relatively high heritability ranging from 0.53 for ARL to 0.88 for SRN. GWA mapping based on BLINK model performing in GAPIT R package allowed us to identify a total of 58 QTLs for all traits. Promising candidate genes were identified for all traits. Two genes, encoding a Serine/threonine-protein phosphatase and a receptor kinases-like protein were identified at qARL-5H.1 and qSRN-5H.3, respectively, and appear to correspond to Arabidopsis orthologs involved in root development

KEYWORDS: Barley, root, root architecture

SIX-ROWED WILD-GROWING BARLEYS ARE HYBRIDS OF DIVERSE ORIGINS

Yu Guo
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)
guoy@ipk-gatersleben.de

Co-Authors:

Axel Himmelbach
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)

Ehud Weiss
Bar-Ilan University, The Martin (Szusz) Department of Land of Israel Studies and Archaeology

Nils Stein
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK); Center for Integrated Breeding Research (CiBreed), Georg-August-University Göttingen

Martin Mascher
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK); German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig

Crop-wild geneflow is common when domesticated plants and their wild relatives grow close to each other. The resultant hybrid forms appear as semi-domesticates and were sometimes considered as missing links between crops and their wild progenitors. Wild-growing barleys in Central and Eastern Asia, named Hordeum agriocrithon, show hallmark characters of both wild and domesticated forms. Their spikes disintegrate at maturity to disperse without human intervention, but bear lateral grains, which were favored by early farmers and are absent from other wild barleys. As an intermediate form, H. agriocrithon has been proposed several times as a progenitor of domesticated barley.
Here, we used genome-wide marker data and whole-genome resequencing to show that all H. agriocrithon accessions of a major germplasm collection are hybrid forms that arose multiple times by admixture of diverse domesticated and wild populations. Although H. agriocrithon barleys have not played a special role in barley domestication, future analysis of the adaptative potential of bi-directional crop-wild gene flow in extant barleys may prove a fertile research field.

KEYWORDS: barley, hybridization, gene flow, domestication, population genomics, plant genetic resources

STUDIES OF BARLEY VIRIDIS-K MUTANTS CONNECT A C-TYPE FERREDOXIN TO CHLOROPHYLL BIOSYNTHESIS

Mats Hansson
Department of Biology, Lund University, Sölvegatan 35B, 22362 Lund, Sweden
mats.hansson@biol.lu.se

Co-Authors:

David Stuart, Malin Sandström, Helmy M. Youssef, Shakhira Zakhrabekova
Department of Biology, Lund University, Sölvegatan 35B, 22362 Lund, Sweden

Poul Erik Jensen
Department of Food Science, University of Copenhagen, Frederiksberg DK-1958, Denmark

David Bollivar
Department of Biology, Illinois Wesleyan University, Bloomington, IL 61702-2900, USA

Ferredoxins are single-electron carrier proteins involved in various cellular reactions. In chloroplasts, the most abundant ferredoxin accepts electrons from photosystem I and shuttles electrons via ferredoxin NADP+ oxidoreductase to generate NADPH or directly to ferredoxin dependent enzymes. In addition, plants contain other isoforms of ferredoxins. Two of these, named FdC1 and FdC2 in Arabidopsis thaliana, have C-terminal extensions and functions that are poorly understood. We identified disruption of the orthologous FdC2 gene in barley (Hordeum vulgare L.) mutants at the Viridis-k locus; these mutants are deficient in the aerobic cyclase reaction of chlorophyll biosynthesis.
The magnesium-protoporphyrin IX monomethyl ester cyclase is one of the least characterized enzymes of the chlorophyll biosynthetic pathway and its electron donor has long been sought. Agroinfiltrations showed that the viridis-k phenotype could be complemented in vivo by Viridis-k but not by canonical ferredoxin. VirK could drive the cyclase reaction in vitro and analysis of cyclase mutants showed that in vivo accumulation of VirK is dependent on cyclase enzyme levels.
The chlorophyll deficient phenotype of viridis-k mutants suggests that VirK plays an essential role in chlorophyll biosynthesis that cannot be replaced by other ferredoxins, thus assigning a specific function to this isoform of C-type ferredoxins.

KEYWORDS: chlorophyll biosynthesis, cyclase, mutants, vir-k, xantha

NAKED BARLEY IN ICELAND

Hrannar Smári Hilmarsson
Agricultural University of Iceland
hrannar@lbhi.is

Spring barley is Iceland's most important cereal, although domestic production is only a fraction of the amount used. Of the total cereal consumption in Iceland only 1% is from domestic production. Since barley is a relatively new crop in Iceland, the barley breeding program has been focused on early heading, straw strength and yield. Less attention has been given on quality, although much data was collected on thousand kernel weight (TKW) and weight by volume, which showed that the quality in Icelandic barley is low. The relatively low quality of barley cultivated in Iceland could explain the demand for imported barley over domestic. In order to increase the quality of barley in Iceland, one strategy was to introgress nakedness into the breeding program. Naked barley corresponds to a specific phenotype of barley where the hull does not adhere to the grain due to the loss of function of the NUD gene. Removing the hull allows to obtain grains with less silica, reducing the harmful and undesirable consequences on human and animal consumption. This mutation leads to an increased protein content in the grain but also makes the embryo more susceptible to damage, since the protective hull is missing. This flaw can be limited by choosing shorter and globular grains. To introgress nakedness into the breeding program, three populations called 368, 369 and 370 were created through a backcrossing scheme using the naked genotype NGB90077 crossed with Arve, a six-row Norwegian cultivar which was popular in Iceland, NGB90077 x ISSkumur, an Icelandic 6-row barley cultivar, and thirdly, a black naked two-row barley Nigrinudum crossed with Golden promise, respectively. Each population was created by backcrossing for three generations. Since the parents of the three populations have different levels of adaptation to the Icelandic environment this should be reflected in the populations where the 369 should be most adapted, followed by 368 and then 370. A selection of 20 individuals from each population, chosen based on maturity and long plump heads, were sown in a field trial along with 39 naked barley genotypes from the USDA GRIN genebank. Results showed that the foreign genebank genotypes were on average faster to reach the stage of awn emergence and on average showed higher TKW. The Icelandic populations ranked for earliness of awn emergence as expected according to their level of adaptation, where 369, the most adapted exhibited awn emergence first, then 368 and last 370. Hence, the presumably non-adapted foreign genebank material performed better under Icelandic conditions than the adapted genotypes. These results suggest that when a novel monogenic trait is introgressed into a breeding program, it might be more efficient to start a new breeding population with parents that contain the gene of interest rather than using the backcross scheme for introgression of novel traits in spring barley. In any case, gene editing would probably be most efficient.

KEYWORDS: Naked barley, hullless, backcross, introgression

MORPHOLOGY, PHENOLOGY, AND DEVELOPMENT

INFLORESCENCE DEVELOPMENT AND FLORAL ABORTION UNDER STRESS IN BARLEY

Maria von Korff
Heinrich Heine University Düsseldorf, Germany //Cluster of Excellence on Plant Sciences "SMART Plants for Tomorrow Needs" Düsseldorf, Germany

maria.korff.schmising@hhu.de

Co-Authors:

Gesa Helmsorig, Tianyu Lan, Meike Siebers, Kumsal Colpan
Heinrich Heine University Düsseldorf, Germany //Cluster of Excellence on Plant Sciences "SMART Plants for Tomorrow Needs" Düsseldorf, Germany

An increase in global average temperature and the occurrence of extreme temperature events threaten crop productivity worldwide. Abiotic stresses, such as heat and drought, are particularly critical during plant reproductive development and affect inflorescence development and morphology, flower fertility and seed set. Barley is characterized by a high degree of genetic diversity and plasticity in response to abiotic stresses. However, the genetic underpinnings of spike development in response to stress are not well understood. We aimed to identify natural genetic variation for developmental plasticity in response to stress in a germplasm collection of elite cultivars and landrace genotypes from the Middle East.
We found that the developmental plasticity and spike development strongly differed between elite and landrace barley genotypes. Using global transcriptome profiling of developing shoot apical meristems under abiotic stress in genotypes with different stress responses we identified genes and gene networks controlling spike development, floret fertility and grain set under stress.
We present and discuss the possible functions of these genes in controlling spike development and fertility in barley.

KEYWORDS: barley, development, abiotic stress

SPIKELET DETERMINACY AS A FACTOR FOR IMPROVING GRAIN YIELD POTENTIAL IN TRITICEAE CROPS

Ravi Koppolu
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)
koppolu@ipk-gatersleben.de

Co-Authors:

Guojing Jiang, Sara G Milner, Twan Rutten, Axel Himmelbach, Yu Guo, Nils Stein, Martin Mascher, Thorsten Schnurbusch
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Correns Strasse 3, OT Gatersleben, 06466 Seeland, Germany

Quddoos H Muqaddasi
BASF Agricultural Solutions GmbH, Am Schwabeplan 8, OT Gatersleben, 06466 Seeland, Germany

Ever since the dawn of agriculture, cereal grains have become the prime source of nutrition for humankind. Both wheat and barley are among the top cereal grain crops cultivated across the globe. Grains in wheat, barley, and other cereals are produced in specialized flower-bearing structures called spikelets. Cereals encompass an amazing source of variation for the spikelet architecture. One such variation is the number of florets (grass flowers that ultimately become grains) produced per spikelet. The barley spikelets bear a single floret; however, wheat spikelets generate more florets per spikelet (often up to four grain bearing florets). Such crucial floret number differences in these crops are manifested by the growth activity or arrest of the spikelet organ called “rachilla”. In the determinate barley spikelets, the rachilla ceases to grow upon initiation of the first floret, whereas in the indeterminate wheat spikelets rachilla growth continues to produce more florets per spikelet making it an important grain yield-determining organ in these crops. Despite its importance, very little is known so far about the genetic and molecular mechanisms driving the rachilla initiation, growth, and arrest processes. Previously, I have characterized a barley indeterminate spikelet mutant, multiflorus 2 (mul2) that quantitatively enhanced rachilla growth and the number of grain-bearing florets per spikelet (up to three). Extensive phenotypic and genetic analysis of barley mul2 uncovered two major quantitative trait loci (QTL) controlling rachilla growth and floret formation. With the knowledge in this research area and available genetic resources, i aim to (i) identify and functionally characterize genes underlying the major rachilla growth/floret formation QTLs in mul2 by map-based cloning, and (ii) discover species-specific genes and gene regulatory networks by targeted transcriptome profiling of rachilla tissues in barley and wheat. The genes underlying the barley mul2.b QTLs and the allelic variation of these genes in wheat will provide an essential genetic tool kit for breeders aiming to improve grain yield potential in these crops. On the other hand, the targeted rachilla transcriptome studies in barley and wheat offer insights into species-specific molecular regulation underlying rachilla development in these crops. Altogether, these findings will provide a comprehensive overview of rachilla development and floret formation in barley, wheat, and probably other cereals; which can be leveraged to improve grain yields in these crops.

KEYWORDS: Barley, wheat, spikelet, floret number

AN INDUCED MUTATION IN HVRECQL4 INCREASES OVERALL RECOMBINATION AND RESTORES FERTILITY IN A BARLEY HVMLH3 MUTANT BACKGROUND

Luke Ramsay
Cell and Molecular Sciences, The James Hutton Institute, Invergowrie, Dundee, Scotland, DD2 5DA, UK
luke.ramsay@hutton.ac.uk

Co-Authors:

Mikel Arrieta, Malcolm Macaulay, Isabelle Colas, Miriam Schreiber
Cell and Molecular Sciences, The James Hutton Institute, Invergowrie, Dundee, Scotland, DD2 5DA, UK

Paul D. Shaw
Information and Computational Sciences, The James Hutton Institute, Invergowrie, Dundee, Scotland DD2 5DA, UK

Robbie Waugh
Cell and Molecular Sciences, The James Hutton Institute, Invergowrie, Dundee, Scotland, DD2 5DA, UK & Division of Plant Sciences, University of Dundee at The James Hutton Institute, Invergowrie, Dundee, Scotland DD2 5DA, UK

Barley breeding is dependent on the biological processes of meiosis and recombination that occur during eukaryotic sexual reproduction. The amount and distribution of recombination represents a potential constraint on the speed and accuracy of selection, in particular when breeding with non-adapted exotic germplasm.
We have attempted to identify genes that limit recombination in barley by performing a suppressor screen for restoration of fertility to the semi-fertile barley mutant desynaptic10 which carries a mutation in HvMlh3. We identified a candidate suppressor line, confirmed its restored fertility and characterised it using a target sequence capture array. We found that this line contained a mutation in the anti-crossover gene RECQL4 resulting in a non-synonymous substitution in the conserved helicase domain.
In subsequent crossing work we were able to demonstrate that there was nearly double the recombination levels in homozygous Hvrecql4 lines compared to wildtype.
Our results confirm the anti-recombination role of RECQL4 in barley and establish the possibility of testing the utility of increasing recombination in the context of traditional crop improvement.

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KEYWORDS: recombination, mutagenesis, meiosis, suppressor, breeding

THE ROLE OF AUXIN IN THE STARCH PRODUCTION OF BARLEY POLLEN

Ivan Acosta
Max Planck Institute for Plant Breeding Research
acosta@mpipz.mpg.de

Co-Authors:

Dhika Amanda, Felix P. Frey, Ulla Neumann, Marine Przybyl
Max Planck Institute for Plant Breeding Research, 50829 Cologne, Germany

Jan Šimura
Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, 90183 Umeå, Sweden

Youjun Zhang, Alisdair R. Fernie
Max Planck Institute of Molecular Plant Physiology, 14476 Potsdam, Germany

Zongliang Chen, Andrea Gallavotti
Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ 08854, USA

Karin Ljung
Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, 90183 Umeå, Sweden

Starch accumulation is a hallmark of maturity and fertility in cereal pollen grains. We present the characterization of the barley mutant male genetic sterile 38 (msg38), which led us to discover that auxin is essential to activate starch accumulation in barley pollen. MSG38 encodes the enzyme HvYUCCA4, catalyzing the last step in the synthesis of bioactive auxin. Importantly, this enzyme is found only in maturing pollen, indicating that pollen grains autonomously produce auxin to drive their maturation.
The accumulation of auxin is required to enhance the expression of central carbon metabolism factors both at the gene and protein level. This results in an increased flux through the canonical pathway that generates energy (ATP) in heterotrophic tissues.
Accordingly, auxin is also necessary to maintain high levels of essential metabolites of this pathway, such as pyruvate (the product of glycolysis), and two tricarboxylic (TCA) cycle metabolites (citrate and pyruvate). Interestingly, the function of HvYUCCA4 seems specific to pollen grains. This suggests HvYUCCA4 as a strong candidate for targeted inhibition of male fertility in barley and wheat. Implementing such a system would enable large scale hybrid seed production between multiple parent pairs, an unfulfilled need of crop breeders.

KEYWORDS: fertility, pollen, starch, auxin, metabolism

SYSTEMATIC COMPARISON OF BARLEY AND WHEAT TRANSCRIPTOMES REVEALS EVOLUTIONARY INSIGHTS OF FLOWER DEVELOPMENT

Vanda B. Marosi
PGSB – Plant Genome and Systems Biology, Helmholtz Center Munich, German Research Center for Environmental Health (GmbH), Neuherberg, Germany
vanda.marosi@helmholtz-muenchen.de

Co-Authors:

Manuel Spannagl, Thomas Lux, Nadia Kamal, Maxim Messerer, Klaus F. X. Mayer, Daniel Lang, Georg Haberer
PGSB – Plant Genome and Systems Biology, Helmholtz Center Munich, German Research Center for Environmental Health (GmbH), Neuherberg, Germany

Bread wheat (Triticum aestivum) and barley (Hordeum vulgare) are two of the most prevalent cereal crops in the world. Understanding the genetic background of their major traits such as flowering is fundamental. The complex processes involved in inflorescence morphogenesis have a crucial impact on crop yield in grasses. Although many transcriptomic studies have been completed focusing on these species individually, the comparison of accompanying traits across such highly identical species remains unraveled.
Utilizing their close evolutionary relationship, we create a cross-species comparative transcriptomic meta-analysis. With various machine learning methods, we examine a comprehensive collection of 1,095 high-quality, public RNA-seq samples from 32 peer-reviewed publications covering 53 different types of tissues. Our orthology based approach establishes comparative gene co-expression networks in the span of four major tissue categories.
We provide examples of evolutionary conserved and diverged gene modules responsible for aspects of flowering in barley and wheat. In addition to our literature mining based validation of genes involved in floral development, we standardized growth-staging methods for grasses, to allow easy sample comparison among distant data collections. As a result, we demonstrate that integration of independent data-sets provides a more robust analysis by covering the expression of rare genes and presents an unbiased approach for systematic identification of trait-specific gene-modules between species.

KEYWORDS: comparative transcriptomics, flower development, cross-species analysis

DISSECTING BARLEY ROOT DEVELOPMENT AND TROPISM BY CLONING CHEMICAL-INDUCED MUTANTS

Silvio Salvi
Department of Agricultural and Food Sciences, University of Bologna, Italy
silvio.salvi@unibo.it

Co-Authors:

Serena Rosignoli, Giuseppe Sangiorgi, Roberto Tuberosa
Department of Agricultural and Food Sciences, University of Bologna, Italy

The molecular genetic basis of root development and tropisms and the extension of their variation in barley germplasm are still poorly understood. Additionally, the exploitation of this knowledge in barley or cereal breeding is very limited. Root growth angle and root depth represent key traits for efficient capture of soil resources, including water and chemical nutrients. By phenotypically screening a chemically induced mutant population (TILLMore, Talamé et al. 2008) in the Morex cv background, we identified 34 root mutants showing altered or defective growth in terms of root length, root morphology, root growth angle and response to gravitropism, roothairs, and other traits. We are genetically mapping and cloning these mutations. Three of these mutants have already been cloned, including two hypergravitropic mutants (enhanced gravitropism1, egt1, and egt2), and a short root mutation (shortroot1, srt1). The genes were cloned by a combination of bulked-segregant analysis and whole genome shotgun sequencing and validation was carried out by TILLING and/or gene editing.
Additionally, we tested for presence of native variation using GWA in a representative barley germplasm collection. Egt2 was shown to encode for a STERILE ALPHA MOTIF domain–containing protein likely involved in executing differential cell elongation in the root elongation zone, independently from auxin. An update about the progress of description and cloning of new root mutants will be reported.

KEYWORDS: Root development, gravitropism, mutant, TILLING

GENETIC ARCHITECTURE OF PHENOLOGICAL AND YIELD-RELATED TRAITS IN A BARLEY POPULATION REPRESENTATIVE OF ELITE BREEDING IN SOUTH AMERICA

Maximiliano Verocai
Departamento de Producción Vegetal, Est. Exp. Mario A. Cassinoni, Universidad de la República, Ruta 3, km 363, Paysandú, Uruguay

mverocai@fagro.edu.uy

Co-Authors:

Silvina Baraibar, Silvia Germán
Instituto Nacional de Investigación Agropecuaria, Estación Experimental La Estanzuela, Ruta 50, Km11, Colonia, Uruguay

Lorena Cammarota
Latitud, LATU Foundation, Av Italia 6201, Montevideo, Uruguay

Fernanda Cardozo
Maltería Uruguay S.A. Ruta 55, Km 26, Ombúes de Lavalle, Uruguay

YLucía Gutiérrez
Department of Agronomy, University of Wisconsin-Madison, 1575 Linden Dr., WI, 53706

Andrés Locatelli
CENUR Litoral Norte–Polo Agroalimentario, Est. Exp. Mario A. Cassinoni, Universidad de la República, Ruta 3, km 363, Paysandú, Uruguay

Fernanda Pardo
Maltería Oriental S.A., Camino Abrevadero 5525, Montevideo 12400, Uruguay

Ariel Castro
Departamento de Producción Vegetal, Est. Exp. Mario A. Cassinoni, Universidad de la República, Ruta 3, km 363, Paysandú, Uruguay

Phenological regulation determines adaptation to specific environments and therefore yield potential and grain quality. Barley production in the Southern cone of South America has a strong dependence on modern European germplasm that combines high grain yield potential and excellent malting quality. This germplasm has limited adaptation to non-optimum environments due to its phenology (late flowering, no photoperiod sensitivity). The goal of our study was to identify chromosome regions and candidate genes associated with yield and phenological traits in South American (specifically Uruguayan) environmental conditions in germplasm representative of the crosses used in local breeding programs. To achieve that we used a nested association mapping population of double haploid lines obtained from crosses between modern European cultivars and local well-adapted germplasm. We studied ten phenological and eight agronomic traits during four consecutive years at four locations in Uruguay in normal (optimum) and late (non-optimum) sowing dates. The population was genotyped by the Illumina barley 50K iSelect SNP array resulting in 6340 informative SNPs covering all chromosomes. We tested various association mapping models and selected a K+PC model as the most appropriate for our study. Our preliminary results indicate a total of 61 QTLs detected, with marker-trait association in all chromosomes except on 4H. Since most of the QTL were found in various environments and were significant for multiple traits, we were able to summarize them in 6 QTL hotspots localized on chromosomes 1H, 2H, 3H, 6H and 7H. Traits detected within a same QTL hotspot were highly correlated. We propose PPD-H1, HvFT2 and Vrn-H3 genes as candidates

for the hotspots located on 2H, 3H and 7H, respectively. Hotspots on 1H and 3H were associated to yield related traits in normal (high potential) and late (non-optimum) sowing dates, which may help to improve yield stability across contrasting environments. Hotspots on 6H and 7H were found only in normal sowing dates and associated to yield related traits and phonological phases highly correlated with yield and grain size. Those genomic regions may help to increase yield potential in optimum environments. The hotspot on 2H (PPD-H1 region) includes QTLs with the higher phenotypic variance of the study. All of them were associated to phenological traits in non-optimum environments. The photoperiod-sensitive allele in the PPD-H1 region reduce the cycle length in late plantings, with the potential of reducing yield and quality loses in more limiting environments thus contributing to wider adaptation under the studied environments. The population used allowed a more direct utilization of the results in breeding. Further association studies analyzing malting quality parameters (beta-glucan content, malt extract, protein content and soluble nitrogen) were also performed in this same population, in an independent work.

KEYWORDS: Association mapping, phenology, grain yield, grain quality, elite germplasm

GENETIC CONTROL OF AWN ROUGHNESS IN BARLEY

Muhammad Awais
IPK Gatersleben, Germany
awais@ipk-gatersleben.de

Co-Authors:

Muhammad Khan, Matthias Jost, Hélène Pidon
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Seeland, Germany.

Nils Stein
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Seeland, Germany.
CiBreed, DNPW, Georg-August University Göttingen, Germany

Awns in cereals are considered to play a significant role in seed protection and dispersal as well as assimilation. Most barley awns are covered with upward-oriented trichomes (barbs) giving them a “rough” surface. On the contrary, barley awns that are lacking such barbs, feel smooth and thus are called smooth awns. The barbs are single-celled trichomes with a silicified cell wall. A major gene (raw1) on chromosome 5H has been isolated, however, one additional locus has been detected by genome-wide association study that contributes to barb formation of barley awns (Milner et al. 2019). To characterize further this putative second locus, F2 mapping populations between “semi-smooth” spring barley cultivar ‘Morex’ and two very smooth composite mutant stocks ‘MHOR597’ and ‘MHOR598’ from IPK genebank, both carrying the recessive semi-smooth allele at the raw1 locus, were used for genetic mapping using genotyping-by-sequencing. A single locus (named raw7HS) could be assigned to a 10 Mbp interval of short arm of chromosome 7H. With the help of improved phenotyping and high-throughput genotyping of 2000 F2 plants, using KASP (Kompetitive allele-specific PCR) markers, we identified a candidate gene that is currently under validation using CRISPR (clustered regularly interspaced short palindromic repeats) based site-directed mutagenesis and gene expression analysis using RT-qPCR. Furthermore, the interaction of both genes (Raw1, Raw7HS) has been studied in the F2 progenies of wild and mutant parents at both loci. The preliminary results demonstrate that the both genes control different characteristics (size, frequency of occurrence) of barb formation. This study will help to determine the molecular mechanism underlying barb formation and to further dissect the correlation of smooth awns and stigma hairiness for the benefit of breeders, farmers and consumers.

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KEYWORDS: Barbs, smooth awns, high-throughput genotyping, KASP, CRISPR

INVESTIGATION OF THE ASSOCIATION BETWEEN BARLEY TWEAKY SPIKE MUTATIONS AND AUXIN BIOSYNTHESIS PATHWAYS USING COMPARATIVE GENOME ANALYSIS

Vėjūnė Pukenytė
Institute of Biosciences, Life Sciences Centre, Vilnius University, Saulėtekis Ave. 7, LT-10257, Vilnius, Lithuania
vejune.pukenyte@gmc.vu.lt

Co-Authors:

Vytautas Čepas, Raimondas Šiukšta
Institute of Biosciences, Life Sciences Centre, Vilnius University, Saulėtekis Ave. 7, LT-10257, Vilnius, Lithuania

Barley is the fourth highest yielding cereal in the world, growing in a wide range of climate and soil conditions due to its natural properties, as well as targeted breeding. Constantly changing environmental conditions demand even more adaptable/fertile varieties, and mutant research is one of the ways to find new genes of interest. The tweaky spike (tw) pleiotropic mutant, developed by chemical mutagenesis, is characterized by alterations in various parts of the spike, increased levels of free radicals and altered amino acid composition in the grains, as well as immunodeficiency and genetic instability. Considering these properties, tw mutants have no direct economic value, but some features of their pleiotropic complex are directly related to fertility and product quality, therefore, the exploration of tw mutation genetic determination and regulation may expand the range of targets for genetic manipulations.

This study aimed to link the phytohormone auxin and tweaky spike mutation and to identify mutations in the auxin biosynthetic pathway that could potentially induce tw phenotype by comparison of tw and Wild Type (WT) WGS data. Two features of tw mutants were selected for phenotype analysis: the presence of the overdeveloped tip of the spike, called a crown, and variation in flower structure. The effect of auxin on tw phenotype was investigated by treating 3-4 leaf stage plants with the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) and observing the resulting changes in flower and spike structures. Treatment with 2,4-D reduced the degree of variation in the number of floral organs, frequency of lodicules transformations and crowned tips compared to control ($P < 0,05$). Interestingly, exogenous 2,4-D induced crowned tips and spikeletless gap formation in the WT ($P < 0,05$). Furthermore, the concentration of indole compounds, including auxin, in the leaves of tw mutants was found to be significantly different from the WT. These findings suggest that auxin quantity alterations in barley spikes could be related to tw phenotype development.

Based on the correlation between exogenic auxin application and tw phenotype, the genetic determination of the auxin biosynthesis pathway in tw mutants was analyzed. Genes involved in auxin biosynthesis in barley were selected by the search of homology with Arabidopsis auxin biosynthesis pathway. Comparative genome analysis between tw2 and WT revealed four deleterious gene variants in the auxin biosynthesis pathway. One of them, HvTSA, was altered in all independent allelic tw mutants and can be considered a gene-candidate that could be responsible for the tw phenotype.

KEYWORDS: 2,4-D, auxin, barley, flower structure, tweaky spike mutant

IDENTIFICATION AND CHARACTERIZATION OF CLV RECEPTORS AND CLE PEPTIDES REGULATING BARLEY SHOOT MERISTEM MAINTENANCE AND DEVELOPMENT

Isaia Vardanega
Department of Developmental Genetics, Heinrich-Heine University, D-40225 Düsseldorf, Germany
isaia.vardanega@hhu.de

Co-Authors:

Gwendolyn Kirschner, Edgar Demesa-Arevalo, Hajer Yousif, Rüdiger Simon
Department of Developmental Genetics, Heinrich-Heine University, D-40225 Düsseldorf, Germany

Ivan Felipe Acosta
Max Planck Institute for Plant Breeding Research, 50829 Köln, Germany

Maria von Korff Schmising
Institute of Plant Genetics, Heinrich-Heine University, D-40225 Düsseldorf, Germany

All the above-ground organs of a plant are developing from a small niche of stem cells called shoot apical meristem (SAM). The balance between maintenance of undifferentiated cells and incorporation of differentiated daughter cells into organ primordia defines SAM size and shape, two qualities that will strongly influence plant architecture and ultimately also seed production. This balance is maintained by the CLAVATA pathway, comprising small secreted CLE peptides that interact with plasma membrane-localized receptor kinases (CLAVATA receptors) to control the expression of WUSCHEL, a transcription factor that acts from the organizing center to promote stem cell fate. This pathway was studied first in Arabidopsis and later in crops plants, such as tomato, corn and rice. Many pathway components are generally conserved among angiosperms, however, the number of genes involved, their expression and function strongly diverge between different plant families. The aim of this project is to identify the key components of the CLAVATA pathway in barley (Hordeum vulgare), with an emphasis on the regulation and function of CLE peptide signals and their receptors.
Barley is the world forth most important crop and has the potential to be the perfect model plant to better understand the complexity of this pathway, due to the organization of its inflorescence, in which the different spikelet meristems are organized in rows displaying a range of developmental stages, but also for its genetic similarity to wheat, its reduced size and its fast life cycle in comparison to other more studied monocots, such as rice and corn.
Putative barley CLV receptors and CLE peptides were identified and targeted by CRISPR-Cas9, in order to understand their gene function and the genetic interactions among them. For detailed expression analysis we perform RNA in situ hybridization and generate fluorescent reporter lines whose expression patterns will be analyzed by confocal microscopy.
We started to analyze the expression pattern and the mutant phenotype of the closest barley homolog of Arabidopsis CLV1 and BAM1 receptors (HvmyCLV1 and HvBAM1). Furthermore, we demonstrated that the barley CLE peptide HvFCP1 is involved in the CLV signaling pathway and we generated and analyzed a transcriptional reporter line of this gene. Following our strategy, we will obtain mutants for key meristem regulators, and reporter lines for CLE peptides and CLV receptors that, combined together, will allow us to study gene functions during barley meristem formation and development.

KEYWORDS: barley development, shoot apical meristem, CRISPR-Cas9, fluorescent reporter lines, CLAVATA pathway

MOLECULAR MECHANISMS OF ASSIMILATE TRANSPORT IN BARLEY GRAINS

Volodymyr Radchuk
Institute of Plant Genetics and Crop Plant Research (IPK)
radchukv@ipk-gatersleben.de

Co-Authors:

Hardy Rolletschek, Ljudmilla Borisjuk
Institute of Plant Genetics and Crop Plant Research (IPK)

In the barley grain, storage reserves accumulate primarily in the endosperm. So, assimilates and nutrients have to pass through numerous tissues of the mother plant before reaching the storage site. Spatially resolved gene expression, metabolite analysis and imaging study have provided valuable information on assimilate allocation in developing grains.
Our approach, based on high-field nuclear magnetic resonance imaging and 13C/1H double resonant coil, demonstrated that nutrients release via the nucellar projection towards the endosperm transfer cells provides an essential mean for the control of seed growth by the maternal organism. Numerous uptake and efflux transporters and metabolic enzymes are active along this route. Based on a comparative analysis of transgenic plants with altered metabolite allocation, we have identified key players involved in the transfer of assimilates in barley grains.
The gene Sugars Will Eventually be Exported Transporter 11b (HvSWEET11b) plays a predominant role in sugar release from nucellar projection into apoplastic space, while Sucrose Transporter 1 (SUT1) is important for sucrose uptake by endosperm transfer cells. It is important to note that HvSWEET11b is able to transport also cytokinins. This dual capacity provides the plant with an efficient means of coordinating the grain's filling, suggesting the synergistic effect of cytokinin and sucrose transport to control grain development.
Vacuolar processing enzymes (VPE2a, VPE2b and VPE2d), encoding the executors of programmed cell death, are exclusively and redundantly transcribed in the nucellar projection. The tissues of nucellar projection revealed a characteristic centripetal gradient in cell degradation. We provide evidence that the cellular disintegration of the nucellar projection is also crucial for the assimilate transfer from maternal seed tissue to the endosperm. Their simultaneous repression resulted in a disturbance to the progression of programmed cell death, thereby delaying the transport of sucrose into the developing endosperm and compromising grain filling. The transgenic effect on grain weight was compensated by transcriptional activation of SWEET genes, leading to a compensatory stimulation of sucrose delivery toward endosperm. We discuss the mechanisms that regulate sugar intake, metabolic re-arrangements and the importance of programmed cell death for grain development. We further demonstrate the necessity of applying an integrative approach for advancing our understanding of molecular physiology of the developing grain.

KEYWORDS: grain, development, assimilate transfer, programmed cell death

NEW BARLEY STRIGOLACTONE MUTANTS IDENTIFIED USING TILLING STRATEGY

Marek Marzec
Faculty of Natural Sciences, University in Silesia, Katowice, Poland
marek.marzec@us.edu.pl

Co-Authors:

Beata Chmielewska, Agnieszka Brąszewska
Faculty of Natural Sciences, University in Silesia, Katowice, Poland

Kaori Yoneyama
Graduate School of Agriculture, Ehime University, Japan

Philip Brewer
ARC Centre of Excellence in Plant Energy Biology, School of Agriculture, Food and Wine, University of Adelaide, Australia

The HorTILLUS (Hordeum-TILLING-University of Silesia) population, developed for spring barley cultivar Sebastian after double-treatment with sodium azide (NaN3) and N-methyl-N-nitrosourea (MNU), was used to identify new strigolactone (SL) mutants. Primers specific for SL biosynthesis genes HvD10 (encoding carotenoid cleavage dioxygenase 8), HvD17 (encoding carotenoid cleavage dioxygenase 7) and HvD27 (encoding β-carotene-9-isomerase) or gene encoding SL repressor (HvD53) were used to screen 6144 individuals from the M2 generation. In total the 61 new alleles were identified (HvD10 - 14; HvD17 - 20; HvD27 - 9; HvD53 - 18).
Homozygous lines carried those alleles were phenotyped in the aspect of tiller number and plant height. Three lines hvd10.d, hvd17.r and hvd27.c exhibited a highly-branched phenotype, characteristic for SL mutants, whereas a mutation in the SL repressor resulted in a low-tillering phenotype of hvd53.f line. Co-segregation of identified mutations with observed phenotype was confirmed using F2 populations obtained after crossing mutants with parent variety Sebastian. In combination with the previously characterized hvd14.d line with a mutation in SL receptor, this collection of SL mutants is a valuable tool for studying the SL role in plant development and adaptation to various stresses.

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KEYWORDS: mutagenesis, strigolactone, TILLING

SPOROPHYTIC CONTROL OF MALE FERTILITY, THE ROLE OF THE HVSWEET4 GENE

Raffaella Battaglia
Council for Agricultural Research and Economics (CREA), Research Centre for Genomics and Bioinformatics, via S. Protaso, 302, I -29017 Fiorenzuola d'Arda, Italy

raffaella.battaglia@crea.gov.it

Co-Authors:

Vania Michelotti, Cristina Crosatti, Monica Colombo, Davide Guerra, Luigi Cattivelli
Council for Agricultural Research and Economics (CREA), Research Centre for Genomics and Bioinformatics, via S. Protaso, 302, I -29017 Fiorenzuola d'Arda, Italy

Roberta Rossi
University of Milan, DiSAA, Via Celoria 2, 20133 Milan, Italy

Aya Kitajima-Koga
Laboratory of Biochemistry, Faculty of Agriculture, Niigata University, Niigata 950-2181, Japan

A vital process for plant growth and differentiation is represented by sugar distribution. A long-distance movement of sugar occurs form source to sink tissues; afterwards, sugar is further distributed following extra-cellular and intra-cellular pathways. Sugars are translocated through symplastic and apoplastic routes, the latter needs the activity of specific transport proteins. The SWEET (Sugars Will Eventually be Exported Transporters) gene family is the most recent identified group of sugar transporters in 2010 (Chen et al.). The presence of this gene family was confirmed in all the eukaryotic kingdoms, and in prokaryotes. Eukaryotic SWEET proteins are characterized by seven predicted transmembrane (TM) domains forming a pore where sugars are expected to move following their concentration gradient.
Focusing the attention on the barley genome, we have identified 22 SWEET sequences which contained the expected TM domains; HvSWEET genes can be further grouped into four clades, as already observed for this gene family in different Angiosperm genomes. It was proposed that belonging to a specific clade correlates with the selectivity toward monosaccharides versus disaccharides (Eom et al., 2015).
Our group is interested in identifying genes that can be manipulated to increase specific yield components. For this reason, we focused the attention on the HvSWEET4 (HvSW4) gene whose maize and rice orthologous play an important role during the seed filling process; both these genes appeared to be recruited during domestication to enhance the hexose import into the developing endosperm (Sosso et al., 2015). In barley, HvSW4 is ubiquitously expressed with a peak of transcription during seed development; furthermore, the HvSW4 protein localizes in the plasma membrane. To highlight the functional role of the HvSW4 gene, we created the sw4 mutant through genome editing in the Golden Promise genetic background. The results indicate that, in barley, the HvSW4 gene is not functionally related to maize and rice orthologues since it is involved in the sporophytic control of male fertility. Morphological analysis of mutant flowers show that early stages of pollen maturation are affected due to lack of activity of the HvSW4 protein, furthermore, mutant allele segregation suggests that HvSW4 acts in sporophytic tissues. Further analyses are currently ongoing to better dissect the molecular pathways influenced by the HvSW4 activity. The effect of an increased dosage of HvSW4 activity is under evaluation in barley lines over-expressing the HvSW4 gene, preliminary data will be presented.

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KEYWORDS: Male fertility, Sugar transport, SWEET, Source-sink

STRATEGIES OF GRAIN NUMBER DETERMINATION DIFFERENTIATE BARLEY ROW-TYPES

Thirulogachandar Venkatasubbu
Institute of Plant Genetics, Heinrich Heine University Düsseldorf, Germany; (Old) Research Group Plant Architecture, Department of Breeding Research, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

Thirulogachandar.Venkatasubbu@hhu.de

Co-Authors:

Ravi Koppolu
Research Group Plant Architecture, Department of Breeding Research, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

Thorsten Schnurbusch
Research Group Plant Architecture, Department of Breeding Research, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany; and Institute of Agricultural and Nutritional Sciences, Faculty of Natural Sciences III, Martin Luther University Halle-Wittenberg, Halle, Germany

Gaining knowledge on intrinsic interactions of various yield components is crucial to improve the yield potential in small grain cereals. It is well known in barley that increasing the grain number (GN) preponderantly improves their yield potential; however, to achieve this aim, it is pivotal to recognize the maximum yield potential (MYP) of the crop. In barley (Hordeum vulgare L.), the MYP is defined as the maximum spikelet primordia number of a spike. Previous barley studies often assumed the awn primordium (AP) stage as the MYP stage regardless of genotypes and growth conditions. From our spikelet-tracking experiments using the two-rowed cultivar Bowman, we found that the MYP stage can be different from the AP stage. Importantly, we find that the occurrence of inflorescence meristem (IM) deformation and its loss of activity coincided with the MYP stage, indicating the end of further spikelet initiation.
Thus, we recommend validating the barley MYP stage with the IM shape and propose this approach (named Spikelet Stop) for MYP staging. Following this approach, we assessed different yield components like potential spikelet number (PSN), spikelet survival (SSL), spikelet number (SN), grain set (GS), and grain survival (GSL), as well as their interactions with GN by using a selected panel of two- and six-rowed barley types. Also, to analyze the stability of these interactions, we performed the study in two growth conditions, greenhouse and field. From this study, we found that in two-rowed, GN determination is strongly influenced by PSN rather than SSL and/or GS in both growth conditions. Conversely, in six-rowed, GN is associated with SSL instead of PSN and/or GS.
Thus, our study exemplified that increasing GN might be possible by augmenting PSN in two-rowed genotypes, while for six-rowed genotypes, the ability of SSL needs to be improved. We speculate that this disparity of GN determination in barley row-types might be due to the fertility of lateral spikelets. Collectively, this study revealed that the GN of two-rowed largely depends on the developmental trait, PSN, while in six-rowed, it mainly follows the ability of SSL.

KEYWORDS: Yield, Grain number, Maximum yield potential, Spikelet survival, Barley

EAM7 ACCELERATES PLANT DEVELOPMENT IN BARLEY BY ALTERING THE EXPRESSION OF CORE CLOCK GENES

Gesa Helmsorig
Heinrich Heine University Düsseldorf
gesa.helmsorig@hhu.de

Co-Author:

Maria von Korff Schmising
Heinrich Heine University Düsseldorf

Photoperiodic flowering is a major factor determining reproductive success and thus yield in plants. Agronomically important temperate cereal crops, such as barley and wheat, are long day plants requiring day lengths above 12 hours to induce reproductive development. While some key players in photoperiodic flowering in crops, such as PHOTOPERIOD 1 (Ppd-H1) have been identified, large parts of the underlying genetic mechanisms are yet mostly uncharacterized and remain to be elucidated.
Barley plants carrying mutations at the early maturity 7 (eam7) locus show accelerated reproductive development and flower in non-inductive photoperiods below 12 hours (short day conditions). This effect is amplified when the mutation appears in combination with the photoperiod-insensitive winter Ppd-H1 allele, implying an interaction between these two loci.
To elucidate the effect of eam7 on gene expression, diurnal expression of circadian clock and clock target genes were analyzed in the wild type, eam7 mutant and double mutant for eam7 and eam1 (Ppd H1). Core circadian clock genes were tested in short day and showed differences in their diurnal expression pattern and intensity in the eam7 mutant. These effects were even more pronounced in the eam7 and eam1 double mutants.
These findings extend our knowledge of the circadian clock in cereal crops and has therefore the potential to improve crop performance in specific environmental conditions.

KEYWORDS: development, circadian clock, photoperiodic flowering

GENETIC MAPPING AND ALLELE DISCOVERY FOR FLOWERING TIME AND PLANT HEIGHT USING A DOUBLE ROUND-ROBIN POPULATION OF BARLEY

Francesco Cosenza
Institute of Quantitative Genetics and Genomics of Plants, Heinrich Heine University, 40225 Düsseldorf, Germany

francesco.cosenza@hhu.de

Co-Authors:

Asis Shrestha, Delphine L. Van Inghelandt, Federico A. Casale, Po-Ya Wu, Marius Weisweiler
Institute of Quantitative Genetics and Genomics of Plants, Heinrich Heine University, 40225 Düsseldorf, Germany

Jinquan Li
Max Planck Institute for Plant Breeding Research, Köln, Germany

Franziska Wespel
Saatzucht Josef Breun GmbH Co. KG, Amselweg 1, 91074 Herzogenaurach, Germany

Benjamin Stich
Institute of Quantitative Genetics and Genomics of Plants, Heinrich Heine University, 40225 Düsseldorf, Germany; Max Planck Institute for Plant Breeding Research, Köln, Germany

Flowering time is one of the factors that determine yield potential and stability in cereals.
Although several genes responsible for controlling flowering time in barley have been described, a comprehensive inventory of its genetic architecture of natural variation for flowering time is lacking. We developed the double round-robin (HvDRR) population of barley from pairwise crosses of 23 spring barley landraces and cultivars. The 4120 recombinant inbred lines (RIL) from 45 sub-populations have been evaluated in multi-environmental trials for their flowering time and plant height.
We observed that the environmental variance, for both characters, was about two to three times bigger than the genotypic variance and therewith was the most important component of the phenotypic variance. Genotype-environment interaction variance was about half the size of the genotypic variance for flowering time and 75 % of genotypic variance for plant height. The single population analysis identified 89 QTLs for flowering time and 80 QTLs for plant height, of which about 5 % have not been described in literature so far, which illustrates the potential of the HvDRR to unravel the genetics of quantitative traits.
Interestingly, we observed that the correlation coefficient between flowering time and plant height ranged from -0.77 to 0.44 in the individual sub-population suggesting an at least partial independent inheritance. This finding was supported by the observation of loosely linked QTL for both characters or allele effects of opposite direction for colocalizing QTL for both characters. Genome-wide sequence and transcriptomes available for all parental inbreds were used to identify candidate genes for the detected QTL.

KEYWORDS: QTL, flowering time, plant height, multi-parent population

PROGRESS TOWARDS QTL MAPPING AND IDENTIFICATION OF MUTANTS FOR CULM-RELATED TRAITS IN BARLEY

Roberta Rossi
Department of Agricultural and Environmental Sciences (DISAA) - University of Milan, Via Celoria, 2, 20133, Milano (Italy)
roberta.rossi1@unimi.it

Co-Authors:

Salar Shaaf, Valerio Gipli, Francesco Nesi, Laura Rossini
Department of Agricultural and Environmental Sciences (DISAA) - University of Milan, Via Celoria, 2, 20133, Milano (Italy)

Alessandro Tondelli, Luigi Cattivelli
CREA Research Centre for Genomics and Bioinformatics, Via San Protaso 302, 29017 Fiorenzuola d'Arda (PC) (Italy)

Silvio Salvi, Serena Rosignoli
Department of Agricultural and Food Sciences - University of Bologna, Viale Fanin 44, 40127, Bologna (Italy)

Ülle Tamm
Estonian Crop Research Institute, J. Aamisepa 1, 48309, Jõgeva (Estonia)

Andreas Maurer, Klaus Pillen
Institute of Agricultural and Nutritional Sciences - Martin Luther University Halle-Wittenberg, Betty-Heimann-Str. 3, 06120 Halle (Germany)

Monika Gajecka, Beata Chmielewska, Justyna Zbieszczyk, Agata Daszkowska-Golec
Institute of Biology, Biotechnology and Environmental Protection - University of Silesia in Katowice, Jagiellońska, 28, 40-032, Katowice (Poland)

Lodging causes significant economic losses by reducing yield and grain quality in cereals. Reduction of plant height has been a major target to increase lodging resistance in barley during the Green Revolution. However, some genes regulating this trait can have undesirable pleiotropic effects. As an alternative strategy, traits associated with culm strength have shown to increase lodging resistance in cereals. However, little is known about the genetic and molecular bases regulating culm architecture traits in barley.
To fill this gap, we have developed segregating populations to further characterize three QTLs for culm morphology that have been previously identified as having stable effects across environments, based on our previous Multi-Environment GWAS on a European barley diversity panel. Towards this objective, we generated two crosses from two large and two small culm cultivars carrying contrasting alleles at the selected QTLs (chromosomes 4H, 5H, and 6H) to characterize their effects and interactions further. For each cross, BC1F1 plants were obtained by using the respective small culm line as the recurrent parent. We identified BC1F1 plants heterozygous at one or more target QTL(s) through an HRM assay, which were subsequently used to develop two Doubled Haploid (DH) populations. These and the respective parents have been genotyped with an Illumina Infinium 15k SNP array to construct linkage maps for QTL analyses using phenotypic data from ongoing trials in different environments.

By scanning the genomic region of the 4H QTL, we identified one candidate gene which is highly expressed in the internodes. A reverse screening of the HorTILLUS mutagenized population led to the identification of four lines carrying mutations at the selected gene. Two of them are being evaluated for their effect on plant and culm development under greenhouse conditions compared to their background cv. Sebastian. In parallel, we are also characterizing two lines showing a large culm diameter and one line with a small culm diameter identified from a forward screening of 57 lines from the TILLMore population obtained by mutagenesis of six-row cv. Morex.

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KEYWORDS: barley, lodging resistance, quantitative trait loci, culm morphology

MERISTEM ESTABLISHMENT AND MAINTENANCE IN BARLEY

Venkatasubbu Thirulogachandar
Institute of Plant Genetics, Heinrich Heine University Düsseldorf, Germany
Thirulogachandar.Venkatasubbu@hhu.de

Co-Authors:

Rüdiger Simon, Yvonne Stahl
Developmental Genetics, Heinrich Heine University Düsseldorf, Germany

Maria von Korff Schmising
Institute of Plant Genetics, Heinrich Heine University Düsseldorf, Germany

Thorsten Schnurbusch
Research group Plant Architecture, Department of Breeding Research, Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

Plant meristems are major determinants of plant architecture, plant diversification, acclimation to environmental stresses, and, more importantly, crop productivity. The goal of the research unit FOR5235 is to further our understanding of meristem establishment, maintenance, and termination in the two major cereal families – maize (Panicoideae) and barley and its close relative, Brachypodium (Pooideae). In this research unit, four groups address the meristem functions of barley shoot and root apices, spikelet, and inflorescence. Collectively, we will identify new molecular players and genetic networks involved in establishing and maintaining various barley meristems.
We will make use of the extensive knowledge on genetic networks controlling meristem fate in the model plant A. thaliana. We will explore the functions of barley orthologs of well-known Arabidopsis molecular players by generating knock-out and ectopic expression mutants of candidate genes like CLAVATA and WUSCHEL RELATED HOMEOBOX. The distribution and signaling of these molecular factors will be established in barley meristems by developing reporter lines of protein candidates and hormones. However, grasses have evolved unique meristems, such as the spikelet meristems in barley, which are not formed by eudicots. Furthermore, several studies have already reported that the functions of Arabidopsis meristem identity genes are not conserved in the grasses.
We will therefore with the help of transcriptomes generated by single-cell RNA-seq or laser captured microdissection methods reveal novel genes and genetic networks controlling meristem functions, namely maintenance of stem cell niches, initiation of organ primordia, identity, and determinacy of inflorescence spikelet meristems. Taken together, the research unit would generate profound knowledge on cereal stem cell systems that can be applied for crop improvements to meet future needs.

KEYWORDS: Meristem establishment, Stem cell, Single cell RNA-seq, Hormones, Genes and gene network

IDENTIFYING MERISTEM-TYPE SPECIFIC REGULATORY CIRCUITS IN COMPLEX INFLORESCENCES OF BARLEYUSING TRANSCRIPTOMICS AT SINGLE-CELL RESOLUTION

Edgar Demesa-Arevalo
Institute for Developmental Genetics, Institute for Plant Genetics and Cluster of Excellence on Plant Sciences, Heinrich-Heine University, 40225 Düsseldorf, Germany

Edgar.Demesa-Arevalo@hhu.de

Co-Authors:

Roisin Ní Chuaig, Isaia Vardanega
Institute for Developmental Genetics, Heinrich-Heine University, 40225 Düsseldorf, Germany

Tobias Lautwein, Karl Köhrer
Biological and Medical Research Center (BMFZ), Genomics and Transcriptomics Laboratory (GTL), Medical Faculty, Heinrich-Heine University, Düsseldorf 40225, Germany

Maria von Korff Schmising
Institute for Plant Genetics and Cluster of Excellence on Plant Sciences, Heinrich-Heine University, 40225 Düsseldorf, Germany

Rüdiger Simon
Institute for Developmental Genetics and Cluster of Excellence on Plant Sciences, Heinrich-Heine University, 40225 Düsseldorf, Germany

Novel transcriptomics technologies with single-cell resolution, allow the study of lesser established model organisms. Such studies are particularly useful in monocot models such as barley and other temperate cereals. In these monocots, the inflorescences are, compared with those of Arabidopsis, considerably more complex and generated by several distinct meristems. These meristem types are the inflorescence meristem (IM), which is the shoot apical meristem (SAM) after floral induction, the triple spikelet meristem (TSM) generated at the flanks of the IM which further develops into the central spikelet meristem (CSM), and two lateral spikelet meristems (LSM). The spikelet meristems give rise to the floral meristem (FM), that generate all floral organs. However, our knowledge of the gene regulatory networks governing the developmental diversity between these meristem types and their developmental trajectories is very limited and mostly focused on a small set of genes that were analysed due to available mutant phenotypes.

The growing spikes of monocots offer a gradient of developmental stages, with the potential to capture all meristem types and their intermediate developmental states (from IM to floret organs). Aiming to better understand these processes, we standardized protocols to isolate protoplasts from barley spikes compatible with two single-cell sequencing platforms based on microfluidics or micro-well plates. These experiments allowed us to sequence 6241 cells arrayed in 17 clusters containing genes known to regulating spike development. Due to the limited availability of marker genes to characterize specific tissues or cell identities in barley meristems, we used Molecular Cartography™ to visualize the gene expression patterns of our candidate genes and clusters at cellular resolution. This novel technology allowed us to simultaneously capture the expression profiles of 100 genes selected from our single cell transcriptomics datasets. Obtaining such detailed spatiotemporal dimension for our gene expression data provided us with important insights into the gene regulatory networks governing spike formation and helped us select potent candidate genes for further functional characterization.

KEYWORDS: Single-cell Sequencing, RNA localization, Spike development, Meristems, transcriptomics

THE MIRNA397A/LACCASE REGULATORY MODULE CONTROL KERNEL SIZE AND SHAPE IN BARLEY

Luigi Cattivelli
CREA Research Centre for Genomics and Bioinformatics, Fiorenzuola d'Arda, 29017, Italy
luigi.cattivelli@crea.gov.it

Co-Authors:

Cristina Crosatti, Andrea Tafuri, Raffaella Battaglia, Katia Gazzetti, Davide Guerra, Erica Mica
CREA Research Centre for Genomics and Bioinformatics, Fiorenzuola d'Arda, 29017, Italy

Working on the functional role of selected miRNAs, we have initially identified the barley miR397a and demonstrated in vitro, through 5'RNA Ligase-Mediated RACE, and in vivo, through ectopic expression, that HvLac12 is a major target of miR397a in barley. Beside a clear down-regulation of Lac12, the ectopic expression of miR397a led to an increased seed length suggesting that the miR397a-laccase module may represent a strategy to increase seed size. Laccases (LACs) are multicopper-containing enzymes potentially involved in the polymerization of phenolic compounds like lignin and evidence, in rice, suggest that Lac genes play a role in the determination of the final seed dimensions. We have therefore induced mutation in HvLac12 to reproduce with genome editing the phenotype highlighted in the ectopic expression of of miR397a. Plant carrying knockout mutations in the second multicopper domain of HvLac12 led to a novel phenotype associated to larger leaves, longer kernels and delayed flowering time.
Taken together our data suggest that miR397a controls kernel size, some development-related traits and, in turn negatively regulates the abundance of the lac12 gene. The functional characterization of key genes acting in this pathway pave the way for gene manipulation in the perspective of boosting yield potential in cereals.

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KEYWORDS: Barley, seed size, laccase

INVESTIGATING THE ROLE OF GRF4 IN BARLEY SEED SIZE DETERMINATION

Luigi Cattivelli
Council for Agricultural Research and Economics (CREA) Research Centre for Genomics & Bioinformatics
luigi.cattivelli@crea.gov.it

Co-Authors:

Katia Gazzetti, Cristina Crosatti, Davide Guerra, Raffaella Battaglia, Erica Mica
Council for Agricultural Research and Economics (CREA) Research Centre for Genomics & Bioinformatics

Growth Regulating Factors are a small family of plant specific transcription factors involved in organ growth and elongation. Among the GRF gene family, GRF4 is the focus of the present research. Beside seed size definition, recent studies in grasses highlight the involvement of GRF4 also in nitrogen assimilation and carbon fixation, thus making it an interesting target for breeding purposes.
In barley, GRF4 is expressed during inflorescence development, with a peak corresponding to the first phases after pollination, then decreasing as kernels develop. As expected, we validated GRF4 as target of miR396 in flowers after pollination. To explore the molecular function of GRF4, we have set up an experimental plan to produce both grf4 mutant lines, generated with a CRISPR/Cas9 approach, and GRF4 over expressing lines.
We have identified three independent lines, over expressing GRF4, producing longer seeds, with an increased seed length ranging from 30% to 50%, whose phenotype was conserved in the progenies. These lines show other pleiotropic phenotypes such as a delayed flowering time and a decreased spike fertility. We are on the process of phenotyping the genome edited mutants, to confirm the role of GRF4 in seed length determination as well as in other aspects of plant growth.

Aknownledgements: This work was supported by Italian Ministry of agriculture through the BIOTECH project.

KEYWORDS: seed-size, GRF, miRNA, CRISPR, yield

A MOLECULAR FRAMEWORK FOR GRAIN NUMBER DETERMINATION IN BARLEY

Yongyu Huang
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, OT Gatersleben, 06466 Seeland, Germany

huang@ipk-gatersleben.de

Co-Authors:

Roop Kamal, Nandhakumar Shanmugaraj, Twan Rutten, Shuangshuang Zhao, Iris Hoffie, Jeyaraman Rajaraman, Yudelsy Antonia Tandron Moya, Mohammad-Reza Hajirezaei, Axel Himmelbach, Jochen Kumlehn, Nicolaus von Wirén, Michael Melzer
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, OT Gatersleben, 06466 Seeland, Germany

Venkatasubbu Thirulogachandar
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, OT Gatersleben, 06466 Seeland, Germany; Institute for Plant Genetics, Cluster of Excellence on Plant Sciences (CEPLAS), Heinrich Heine University, 40225 Düsseldorf, Germany

Götz Hensel
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, OT Gatersleben, 06466 Seeland, Germany; Centre for Plant Genome Engineering, Institute of Plant Biochemistry, Heinrich Heine University, 40225 Düsseldorf, Germany

Naser Poursarebani
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, OT Gatersleben, 06466 Seeland, Germany; Hudson River Biotechnology, Nieuwe Kanaal 7V, 6709 PA Wageningen, Netherlands

Udda Lundqvist
Nordic Genetic Resource Center, Alnarp SE-23053, Sweden

Nils Stein
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, OT Gatersleben, 06466 Seeland, Germany; Center for Integrated Breeding Research (CiBreed), Georg-August-University Göttingen, Göttingen, Germany

Martin Mascher
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, OT Gatersleben, 06466 Seeland, Germany; German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

Thorsten Schnurbusch
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, OT Gatersleben, 06466 Seeland, Germany; Martin Luther University Halle-Wittenberg, Faculty of Natural Sciences III, Institute of Agricultural and Nutritional Sciences, 06120 Halle, Germany

Flowering plants with indeterminate inflorescences often produce more floral structures than they actually require. In barley (Hordeum vulgare L.), only few of the floral primordia initiated before flowering do eventually develop into grains, potentially representing an untapped yield potential. In this study, we systematically dissected the composition of barley grain number determination under controlled glasshouse conditions. We showed that the variation in spikelet primordia number only accounts for ~57% and ~11% of final spikelet and grain number variations, respectively. Our genetic study demonstrates that flowering-time genes dominate spikelet primordia initiation by altering the phase duration. However, when excessive primordia are present, an abortion penalty occurs that hinders the production of more grains. By combining genetic analysis, gene expression profiling, metabolic measurements and histological observations, we further showed that barley spikelet growth is specified by chloroplast and vascular developmental programs orchestrated by a CCT MOTIF FAMILY4 (HvCMF4) gene expressed in the inflorescence vasculature. Mechanistically, HvCMF4 acts in connection with the vascular-localized circadian clock to control greening of the neighboring tissues, which autotrophically sustain floral growth. Evolutionarily, CMF4 is specific to the Pooideae subfamily (including wheat, barley, rye and Brachypodium) of the grasses, which all feature early inflorescence greening compared with other grass species, such as rice and sorghum. This suggests that early inflorescence greening might be an evolutionary innovation for the Pooideae species and that neofunctionalization of CMF4 has been essential for it. Moreover, we show that natural HvCMF4 variants are associated with environmental adaptation and spikelet survival. Notably, stacking beneficial natural alleles for both primordia number and survival provides positive implications on grain production. Our findings provide insights into the molecular underpinnings of grain number determination in cereal crops.

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KEYWORDS: circadian clock, chloroplast, vasculature, spikelet initiation, spikelet growth, barley adaptation

GENETIC ASSOCIATION OF SPIKELET ABORTION WITH SPIKE, GRAIN, AND SHOOT TRAITS IN HIGHLY-DIVERSE SIX-ROWED BARLEY (HORDEUM VULGARE L.)

Roop Kamal
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstraße 3, D-06466 Seeland OT Gatersleben, Germany

kamal@ipk-gatersleben.de

Co-Authors:

Thorsten Schnurbusch, Yusheng Zhao
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstraße 3, D-06466 Seeland OT Gatersleben, Germany

Quddoos H. Muqaddasi
European Wheat Breeding Center, BASF Agricultural Solutions GmbH, Am Schwabeplan 8, D-06466 Seeland OT Gatersleben, Germany

Spikelet abortion (SA) in barley is a phenomenon in which a few spikelet primordia, after reaching the maximum yield potential stage (MYP)—a stage after which no new spikelet primordia are produced on an immature spike—start to abort. Regardless of the row-type, both apical and basal SA occurs, and their extent decides the number of grain-bearing spikelets retained on the spike—thus, affecting the yield potential of the barley spike. Therefore, understanding the molecular and genetic mechanism(s) of SA and altering their extent, represent an opportunity to increase barley yields. Here, we investigated the variation for apical SA along with 16 major spike, shoot, and grain traits in a panel of 417 six-rowed spring barleys. Our analyses showed a significantly large genotypic variation resulting in high heritability estimates for all the investigated traits. Among the seven spike-related traits, SA was negatively correlated with final spikelet number (FSN), spike length and density, while positively with awn length. The positive correlation indicates that rapidly growing awns act as a competitor (sink competitor) of the developing spikelets for the available resources during the early spike growth phase. Our path analysis revealed that potential spikelet number (PSN) and FSN explain 93% of the observed phenotypic variability for SA, with PSN behaving as a suppressor trait that magnifies the effect of FSN. In addition, SA also showed a moderate positive correlation with grain length and area. Our hierarchical clustering revealed distinct genetic underpinning of grain traits from the spike and shoot traits. Geographical origin also showed associations with the traits where European accessions displayed higher SA and grain and shoot trait values, whereas the trend was opposite for the Asian accessions. Asian accessions displayed the lowest SA, indicating the presence of favorable alleles that may be exploited in breeding programs. The genetic interactions among traits suggest novel breeding targets and easy-to-phenotype “proxy-traits” for high throughput on-field selection for grain yield, especially in early generations of barley breeding programs. Based on a large set of diverse barley accessions, our results provide a deeper understanding of quantitative genetic nature of SA, its association with traits of high agronomic importance, and a resource for further genetic mapping studies.

KEYWORDS: Awn length, final spikelet number (FSN), , grain traits, potential spikelet number (PSN), spikelet abortion (SA)

A CHEMICALLY-INDUCED NEW MUTATION AFFECTING LEAF WIDTH IN BARLEY

Serena Rosignoli
Department of Agricultural and Food Sciences, University of Bologna, Italy
serena.rosignoli2@unibo.it

Co-Author:

Silvio Salvi
Department of Agricultural and Food Sciences, University of Bologna, Italy

Leaf blade area, including leaf length and width components, is a complex trait strategic for improving yield and adaptation as it affects photosynthesis and response to water stress.
Previous studies enabled to map QTLs for leaf blade area in spring barley (Alqudah et al. 2018), to find pleiotropic effect on leaf length and width of Pdp-H1 in winter barley (Digel et al. 2016) and to identify the gene Broad leaf 1 (Blf1) affecting leaf width (Jöst et al., 2016).
In this study, a field-based phenotypic screening for leaf phenotypes of the barley TILLMore population (Talamè et al. 2008, cv. Morex background, chemically mutagenized using NaN3, currently including approx. 3,600 M6 lines) provided a mutant line, TM2544, displaying an obvious 'broad leaf' phenotype compared to Morex wt. Phenotypic comparisons showed an average leaf width increase of 71% in the field and 64% in pots of TM2544 compared to Morex, occurring from the fourth leaf upwards.
We outcrossed TM2544 with cv Barke and obtained an F1 population with 100% of broad leaf phenotype. In the F2 generation we observed a 3:1 segregation for the mutant phenotype confirming the monogenic dominant control of the trait. We whole-genome shotgun sequenced TM2544 and identified 62 functional mutations, however a strong candidate gene remains to be identified. TM2544 does not carry any functional mutations in Blf1 and a formal complementation test is in progress. We are currently carrying out the genetic mapping and cloning of TM2544 new broad leaf gene using a mapping by sequencing approach.

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KEYWORDS: leaf, mutants, Blf1, BSA-Seq

DISSECTION OF LIFE HISTORY TRAITS IN ANNUAL AND PERENNIAL WILD RELATIVES OF BARLEY

Ilyse Putz
Heinrich-Heine-Universität Düsseldorf; CEPLAS
ilyse.putz@hhu.de

Perennial crops that grow over many seasons and require low agricultural input represent a paradigm shift in modern agriculture and hold great potential for sustainable production systems. However, developing perennial crops is time-consuming and challenging because of hybridization barriers and the difficulty to combine longevity with high grain yield in perennial plants. To increase the speed of breeding perennial crops, new genome editing techniques, like CRISPR-Cas, could be utilized. However, this requires a better understanding of the genetic underpinnings of annual versus perennial growth. In this study, I use annual and perennial barley wild relatives as the genetic resource to investigate annual perennial life history traits and to identify genetic variants which could be used for the targeted engineering of perennial barley. First, I have developed a number of interspecific hybrid crosses by crossing annual and perennial Hordeum species. In a first succession of sowing, I obtained five interspecific hybrid plants, and I have scored traits such as flowering time, duration of flowering, and senescence. The seed set of the hybrids has been hindered by hybridization incompatibility such as unsynchronized development of floral organs and increased vegetative senescence. In addition, I phenotype different associated life history traits such as juvenility and duration of flowering in these species. Furthermore, I analyse genetic differences linked to annual versus perennial growth via RNA-sequencing in parental and hybrid plants to identify regulatory variation between annual and perennial plants. This will be the first approach in identifying genetic variation underlying different life history traits in the Hordeum species.

KEYWORDS: RNA-sequencing, hybridization, crop wild relatives, perennial-annual

CRONOBARLEY 2.0: A SIMPLE MODEL TO PREDICT PHENOLOGY IN MALTING BARLEY BASED ON CULTIVAR THERMO-PHOTOPERIODIC RESPONSE

Santiago Alvarez Prado
University of Buenos Aires, School of Agriculture; Actual address, University of Rosario
psalvare@agro.uba.ar

Co-Authors:

Betina Kruk, Mariana Jardon, Maria Elena Fernandez Long, Anibal Crespo, Daniel Miralles
University of Buenos Aires, School of Agriculture

Alan Severini
INTA Pergamino

In barley as many other cereals crops, heading time is an agronomically critical feature determining crop performance, as it defines the environmental conditions along the crop cycle, and especially during the critical period around flowering for yield determination. Therefore, it is crucial to understand how environmental factors affect barley phenology for predicting the different phenological stages when crop is exposed to different environments. Crop simulation models are useful tools for predicting crop growth and adjusting management practices to current environmental conditions. Despite advances in phenology prediction, most simulation models are complex and require multiple inputs and specific system requirements to get an accurate prediction, which makes it hard for the massive use by farmers, agronomic advisors and agronomists in general. The objective of this study was to design and calibrate a simple crop phenology prediction model using photoperiod-corrected thermal time sums. To fulfill this objective, 22 commercial barley cultivars were sown under field conditions in five contrasting sowing dates during 2018 and 2019 growing seasons in Buenos Aires and Bordenave. During the crop cycle, phenological stages of sowing, emergence, beginning of stem elongation, awns appearance and heading and physiological maturity were determined. Thermal time was determined using a base temperature of 0°C for all stages except during the grain filling where a base temperature of 7.5°C was used. The physiological model was used to predict heading dates for each genotype using parameters as estimated by the corrected thermal time. For model validation, independent datasets were collected from the national barley trial network from different sites across the Argentinean barley belt region covering a wide range of environments using data from the last 5 year. To assess the model outputs the RMSE between observed and predicted heading dates was determined, the bias of the prediction in days, a slope test between the observed/predicted heading date slope and the 1:1 regression to determine the model capacity to predict accurately. Results showed that in all genotypes cycle duration between emergence and heading time was shortened as sowing was delayed. The rate of shortening by each day of delay in sowing varied among the genotypes and for different phenological stages. For instance, the rate of shortening for the period emergence-beginning of stem elongation ranged from -1.12 to -2.93 °Cd day-1. For the period emergence-awns appearance the rate of shortening varied among the cultivars from -2.45 to -4.34 °Cd day-1. Model validation in 20 different locations showed a RMSE of 6.8 days, nRMSE 2% and a bias of 2.04 days. In summary, the model showed an accurate prediction of barley phenology for the large number of genotypes used in the present study. The algorithms were incorporated in the web-based application named CRONOCEBADA (i.e. CRONO BARLEY in English)

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KEYWORDS: Phenological stages, modelling, barley

IDENTIFICATION OF GRAVITY REGULATED GENES THAT ENCODE DIRECT INTERACTION PARTNERS OF BARLEY ENAHANCED GRAVITROPISM 2

Li Guo
University of Bonn
lguo@uni-bonn.de

Co-Authors:

Silvio Salvi
University of Bologna

Frank Hochholdinger
University of Bonn

Plant root gravitropism allows plants to adjust the direction of their root growth, thereby anchoring the aboveground of plants and optimizing the uptake of water and nutrients. Root gravitropic response can be divided into three steps that occur in different zones of the root tip. First, gravity perception takes place mainly in the root cap. Second, signals are transmitted from the root cap to the elongation zone and finally result in a curvature response in the elongation zone. In a previous study, we cloned the barley ENHANCED GRRAVITROPISM 2 (EGT2) gene that controls root gravitropism (Kirschner et al., 2021). In the present study, we revealed genes regulated by EGT2 by tissue-specific comparative transcriptomic analysis of wild-type (WT) and egt2 roots in a time course experiment between 0 and 12 h after rotation. A principal component analysis revealed greater transcriptomic differences between tissues than between genotypes or the time of gravistimulation. Pairwise comparisons between egt2 and WT transcriptomes as well as WT transcriptomes over the time course indicated that the elongation zone contained more differentially expressed genes, whereas the meristematic zone had the fewest differentially expressed genes at all time points. Of these, 357 genes were significantly differentially regulated (FDR<5% and $|log2FC| \geq 1$) in both egt2 and gravistimulated WT roots at ≥1 time point, indicating their potential roles in regulating the root gravitropic response of the mutant egt2. Yeast-two-hybrid screening revealed, that 24 genes preferentially expressed (FDR < 5%) in the elongation zone of gravity-stimulated WT roots encode interaction partners of EGT2. Moreover, 16 genes preferentially expressed (FDR < 5%) in the elongation zone of egt2 encode interaction partners of EGT2. Taken together, this study provides insights into the transcriptome reprogramming of barley root gravitropic response and identified graviregulated genes that encode direct interaction partners of EGT2.

KEYWORDS: EGT2, gravitropism, RNA-seq, yeast-two-hybrid, barley

PPD-H1 ALLELES AND REPRODUCTIVE OUTPUT IN BARLEY

Jorge Damian Parrado Marrades
Department of Crop and Forest Sciences, University of Lleida – AGROTECNIO- CERCA, Av. Rovira Roure 191, 25198 Lleida, Spain

jorge.parrado@udl.cat

Co-Authors:

Roxana Savin
Department of Crop and Forest Sciences, University of Lleida – AGROTECNIO- CERCA, Av. Rovira Roure 191, 25198 Lleida, Spain

Gustavo A. Slafer
Department of Crop and Forest Sciences, University of Lleida – AGROTECNIO- CERCA, Av. Rovira Roure 191, 25198 Lleida, Spain. ICREA, Catalonian Institution for Research and Advanced Studies, Spain

Flowering time in barley is critical for adaptation, and VRN-H1 and PPD-H1 are the major genes controlling it through vernalization and photoperiod sensitivity, respectively. They are known to affect the duration of pre-anthesis phases when numerical components of yield are being produced. Consequently, these genes have been reported to affect not only phenological development, but also spike fertility (normally, the longer the time to flowering, the greater the number of fertile florets per spike). As direct effects of PPD-H1 on spike fertility have not been shown, we aimed to determine if the allelic variation of PPD-H1 (in two contrasting VRN-H1 backgrounds) also affect floret development and spike fertility beyond the effects on phenology. We grew four near isogenic lines (NILs) of barley (produced by CSIRO, Australia) to study the performance of vernalized plants carrying different allelic combinations of PPD-H1 and VRN-H1 genes under either natural short day or 24 h daylength both under field (artificially extending the day with low-intensity lamps) as well as in a chamber experiment at relatively realistic low temperature (12 ºC) under 12 or 24 h photoperiod. Under short days, reduced photoperiod sensitivity lines (ppd-H1) showed a longer time to flowering in both VRN-H1 backgrounds. The longer the duration to anthesis (due to genetic factors) the greater the spike fertility. The extended photoperiod treatment resulted in thermal time to anthesis to be relatively uniform across lines. In this condition, without relevant changes in time to flowering, a greater number of fertile florets was observed in the reduced photoperiod sensitivity (ppd-H1) genotype carrying either Vrn-H1 (spring) or vrn-H1 (winter) backgrounds. In all cases, the greater number of fertile florets per plant was better explained by the number of fertile florets per spike than by the number of spikes per plant. Under short days, the differences in spike fertility seemed to be related to the maximum number of florets initiated (winter background) or the survival of initiated floret primordia (spring background). In extended photoperiod, no difference in the maximum number of differentiated florets was observed, the survival of initiated floret primordia being the main cause of the increased number of fertile floret. Naturally the central spikelets did not show differences in floret development, however ppd-H1 induced greater development of less favored apical florets than PPD-H1. As the effect was evidenced even in absence of major effects on time to anthesis, it seems to be constitutive, through promoting a greater survival of floret primordia, in turn increasing spike fertility.

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KEYWORDS: Hordeum vulgare, spike fertility, photoperiod, vernalization

CELL NON-AUTONOMOUS SIGNALING BY AN ALOG (HVALOG1) TRANSCRIPTION FACTOR SPECIFIES THE DETERMINACY OF THE BARLEY TRIPLE-SPIKELET MERISTEM

Guojing Jiang
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Corrensstr. 3, 06466 Seeland, Germany

jiangg@ipk-gatersleben.de

Co-Authors:

Twan Rutten, Ravi Koppolu, Götz Hensel, Yongyu Huang, Yudelsy Antonia Tandron Moya, Jeyaraman Rajaraman, Naser Poursarebani, Nicolaus von Wiren, Jochen Kumlehn, Martin Mascher, Thorsten Schnurbusch
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Corrensstr. 3, 06466 Seeland, Germany

A typical feature of the barley inflorescence is the spikelet-triplet growing from one rachis node. It is mainly defined by the triple-spikelet meristem (TSM) that differentiates from the spikelet ridge after reproductive transition. However, the mechanisms underlying differentiation and maintenance of the TSMs remain elusive. Here, we examined a recessive mutant previously named extra floret-a (flo.a). Detailed phenotypic analysis showed that the phenotypes of the flo.a mutant rely on two aspects: (i) production of extra spikelets adaxial to the primary central spikelets starting from the upper-mid portion to the tip of the spike; and (ii) modulation of inner and outer glume boundary establishment, resulting in a fused leaf-like organ.
FLO encodes an ALOG1 transcription factor (HvALOG1), a close homolog of Arabidopsis thaliana LSH1 and Oryza sativa G1. ALOG genes have been shown to be involved in light signaling, floral organ specification, spikelet development and inflorescence branching in several plant species.
By ectopic expression of a HvALOG1-p::HvALOG1:GFP construct, we show that HvALOG1 is not expressed in the reproductive meristem during early spike development but expressed at the base of the floral organ primordia after spikelet initiation. Transcriptome profiling revealed interactions of HvALOG1 with other known regulators of inflorescence architecture. In particular genes involved in boundary formation, meristem maintenance and organization exhibited opposite expression patterns to those involved in auxin-dependent signaling pathways and organ development. Auxin accumulation in the upper-middle spike of flo.a mutant appears to promote extra spikelet formation and modulate glume boundary establishment. We propose that HvALOG1 provides signals from the meristem boundaries non-autonomously to specify the determinacy of the barley TSMs; however, the boundary establishment between the two glumes appears to be cell autonomous. Furthermore, phylogenetic analysis showed that ALOG family members share a conserved domain and can be distinguished into different lineages, suggesting independent evolutionary events in eudicots and grasses. The ten barley ALOG members were divided into three clades, eight of which were specifically expressed in young spikes. These results provide evidence that the positional effect of the flo.a phenotypes within spikes is derived from the functional redundancy of other ALOG family members during early spike development.

KEYWORDS: ALOG, Extra spikelet, Triple-spikelet meristem, Functional redundancy

CHARACTERIZATION OF BARLEY PLANT ARCHITECTURE MUTANTS FROM THE HORTILLUS POPULATION

Laura Rossini
Department of Agricultural and Environmental Sciences (DISAA) - University of Milan, Via Celoria, 2, 20133, Milano (Italy)
laura.rossini@unimi.it

Co-Authors:

Salar Shaaf, Abhisek Biswas, Nunzio Cicero, Alessandro Vajani, Carlotta Claudia Ferrario
Department of Agricultural and Environmental Sciences (DISAA) - University of Milan, Via Celoria, 2, 20133, Milano (Italy)

Veronica Gregis, David S. Horner, Matteo Chiara, Marta Mendes, Alex Cavalleri
Department of Biosciences - University of Milan, Via Celoria, 26, 20133, Milano (Italy)

Menachem Moshelion, Ahan Dalal
The Hebrew University of Jerusalem, Robert H. Smith Institute for Plant Sciences and Genetics in Agriculture, Rehovot 7610001 (Israel)

Alessandro Tondelli
CREA Research Centre for Genomics and Bioinformatics, Via San Protaso 302, 29017 Fiorenzuola d'Arda (PC) (Italy)

Beata Chmielewska, Agnieszka Janiak
Institute of Biology, Biotechnology and Environmental Protection - University of Silesia, Jagiellońska, 28, 40-032, Katowice (Poland)

In grasses, plant architecture traits such as tiller number, leaf size and orientation play a major role in plant productivity and adaptation to the environment and understanding of their genetic basis provides a foundation for breeding improved varieties. Seeking for genes associated to these traits in barley, our group is applying forward and reverse genetic screenings on TILLING populations such as HorTILLUS, obtained by chemical mutagenesis of spring two-row cultivar Sebastian.
Based on field evaluations in two different environments, we identified two lines exhibiting more erect leaf angle compared to the Sebastian background (Aghdam et al. 2021 https://doi.org/10.1007/s42976-021-00178-6): mapping of the respective loci on chromosomes 5H and 7H via exome-sequencing/SNP array Bulk Segregant Analysis provided a starting point for the identification of candidate genes.
In parallel, we are characterizing mutants altered in the pathway of strigolactones, a class of phytohormones that emerged as key players in plant architecture and abiotic stress responses in rice and other species. Allelic variants of a barley strigolactone pathway gene were identified from reverse screening the HorTILLUS population and phenotypic analyses suggest a role for this gene in shoot and root architecture. The performance of these lines under different water availabilities is under investigation at the PlantArray physiological phenotyping platform and complementation of the corresponding Arabidopsis mutant is underway to test for functional conservation between the two species.
The selected mutants represent promising materials to better understand the molecular mechanisms controlling plant architecture in barley.

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KEYWORDS: Plant architecture, leaf angle, strigolactones, TILLING

CLONING AND FUNCTIONAL CHARACTERIZATION OF THE BARLEY VIRIDIS ZB63 PHOTOSYNTHETIC MUTANT

Alessandro Tondelli, Cristina Crosatti
CREA-Research Centre for Genomics and Bioinformatics, Fiorenzuola d'Arda, Italy
alessandro.tondelli@crea.gov.it

Co-Authors:

Viola Torricella, Luca Tadini, Lisa Rotasperti, Paolo Pesaresi
Department of Biosciences, University of Milan, Milan, Italy

Davide Guerra, Luigi Cattivelli
CREA, Research centre for Genomics and Bioinformatics, Fiorenzuola d'Arda, Italy

Nils Stein
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Seeland, Germany

Barley genetic stocks offer a unique collection of chloroplast-deficient mutants that represent an important resource to explore chloroplast biogenesis and photosynthetic molecular mechanisms. Most of these mutants have been genetically and biochemically characterized in the past, among them the photosynthetic mutant viridis zb63 is a lethal mutation with pale green leaves. Biochemical studies have demonstrated that zb63 contains a functional PSII with a minimal antenna system and a depleted PSI, a condition leading to the constitutive reduction of plastoquinone even when grown at very low light intensities. No other allelic mutations for this locus have been reported so far. The zb63 mutation was initially localized in the region between 40 and 60 Mb of chromosome 2H following a BSA carried out on a BC3F2 mapping population using an exome capture and sequencing approach. Then, about 480 BC3F2 individuals have been genotyped with KASP and CAPS markers to narrow-down the mutation to the physical interval between 43 and 45 Mb. The 22 genes annotated in this region on the reference sequence of the cultivar Morex were further studied in silico, and a gene coding for Pentatricopeptide Repeat (PPR) protein was identified as possible candidate. The gene carries a 14 bp deletion in exon 1 that creates a premature stops codon. In Arabidopsis, a knockout in the orthologous Pdm4 gene leads to albina phenotype with completely white leaves. The PPR gene was transiently silenced through Virus Induced Gene Silencing and the obtained leaves expressed the typical viridis phenotype of the zb63 mutant. Furthermore, northern and western-blot assays confirmed that silencing the PPR candidate gene reproduces a molecular phenotype identical to zb63 mutant. The cloning of the zb63 mutant will shade light on mechanisms controlling the PSI assembly.

KEYWORDS: viridis, zb-63, photosynthetic mutants, PPR

GENETIC DISSECTION OF CHLOROPLAST BIOGENESIS IN BARLEY

Mingjiu Li
Department of Plant Sciences, University of Oxford
mingjiu.li@plants.ox.ac.uk

Co-Authors:

Götz Hensel,
Center for Plant Genome Engineering, Institute of Plant Biochemistry, Heinrich Heine University Düsseldorf

Ganggang Guo
Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China

Hélène Pidon
Institute for Resistance Research and Stress Tolerance of the Julius Kühn-Institute

Alberto R. Prina
Institute of Genetics 'Ewald A. Favret' (IGEAF), INTA CICVyA/Argentina, Hurlingham, Buenos Aires, Argentina

Michael Melzer, Martin Mascher, Jochen Kumlehn, Nils Stein
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Seeland, Germany

Thomas Börner
Molecular Genetics, Institute of Biology, Humboldt University, Berlin, Germany

Photosynthesis converts solar energy into chemical energy which provides the initial driving force for human activities. In land plants, photosynthesis occurs in an autonomous organelle called chloroplast. The biogenesis of chloroplast is dependent on the coordinate expression of genes encoded in both nuclear and plastid genomes. The barley chlorophyll mutant legacy provides as a valuable genetic resource for dissecting nuclear-encoded genes that in control of chloroplast development. Here, I am going to report on cloning of the albostrians (HvAST / HvCMF7) and luteostrians (HvLST / HvClpC1), of which mutation causing a green-white and green-yellow striped phenotype, respectively. The HvCMF7 gene encodes a CCT MOTIF FAMILY protein targeting to chloroplast. In contrast, HvClpC1 encodes an ATP-dependent Clp protease subunit C1. Notably, in order to circumvent the recombination-dependent labor-intensive map-based cloning approach, we demonstrated how a combination low-resolution genetic mapping, whole-genome resequencing and comparative functional analyses provides a promising path toward candidate identification of genes involved in plastid biology and / or photosynthesis, even if genes are located in recombination poor regions of the genome.

KEYWORDS: Chloroplast biogenesis, barley chlorophyll mutants, fast gene isolation, forward genetics, reverse genetics

IMPROVE GENETIC GAIN THROUGH THE UNDERSTANDING OF GENOTYPE BY ENVIRONMENT INTERACTIONS IN SPRING BARLEY BREEDING PROGRAMS

Maëva BICARD
Institut Polytechnique UniLaSalle, AGHYLE (SFR Condorcet FR CNRS 3417), 19 rue Pierre Waguet, 60000, Beauvais, France & Kronenbourg SAS, Boulevard de l'Europe, 67210, Obernai, France

maeva.bicard@unilasalle.fr

Co-Authors:

Guénolé BOULCH, Michel-Pierre FAUCON, Bastien LANGE
UniLaSalle, AGHYLE (SFR Condorcet FR CNRS 3417), 19 rue Pierre Waguet, 60000, Beauvais, France

Dominique VEQUAUD, Pierre PIN
SECOBRA Recherches, Le Bois Henry, 78580, Maule, France

Genotype by environment interactions (GEI) observed in multi-local and multi-annual trials are slowing down the genetic progress in spring barley breeding programs. Crop growth models are powerful tools to help breeders unravelling the origins of these interactions and design better breeding strategies to mitigate their effects. The objectives of the project are to (i) determine environmental factors driving GEI in spring barley, to (ii) build an environmental classification based on the main GEI-drivers, and to (iii) define a high-throughput criterion to select for GEI-drivers. Based on a two-year connected trial network in Northern Europe, phenology, growth and yield parameters of six cultivars will be recorded. Accurate simulations will be developed using the Decision Support System for Agrotechnology Transfer (DSSAT) CERES barley-model by combining these trial data to meteorological records and soil physiochemical parameters. Ecoclimatic factors, i.e. climatic variables calculated between two developmental stages, will be calculated on a multi-year trial database prior to be analyzed to detect the main GEI-drivers. Target Population of Environments (TPE) will be defined based on the frequency of each environmental class in Northern Europe. The environmental classification will allow balancing trial results to improve breeding decisions. Finally, drones will be used to capture plant traits that would be predictors of the cultivar response to the main GEI-drivers. The identification of high-throughput criteria to select for GEI-drivers will provide a relevant and rapid screening method to maximize the genetic progress in spring barley breeding programs.

KEYWORDS: Cereals, Climatic factors, Crop modelling, Genotype by environment interactions, High-throughput phenotyping, Spring barley

BALTIC AND NORDIC BARLEY: A REGIONAL PERSPECTIVE

TOGETHER WE ARE STRONGER - UNITED FORCES TO PROMOTE NORDIC PRE-BREEDING IN SPRING BARLEY

Therese Bengtsson
Swedish University of Agricultural Sciences
therese.bengtsson@slu.se

Co-Authors:

Ahmed Jahoor
Nordic Seed A/S

The PPP Barley Consortium
Swedish University of Agricultural Sciences, Nordic Seed A/S, Agricultural University of Iceland, Graminor AS, Boreal Plant Breeding Ltd, Natural Resources Institute Finland, Sejet Plant Breeding,Lantmännen ek. för., Copenhagen University

Pre-breeding for broadening the genetic base and introducing genes for tolerance/resistance towards abiotic and biotic stress in an adapted background is a long-term process that requires large resources. Currently, large multi-national companies carry out most of the global plant breeding, neglecting the small and unique market of the Nordic region. With that in mind, the Nordic Council of Ministers (NMR), 2011 initiated a framework for public-private partnerships (PPP) in pre-breeding. Financed 50-50 by NMR and through in kind from the breeding entities. The purpose of the PPP was to promote the small breeding entities in the Nordic countries by creating a foundation for long-term pre-commercial collaboration.
One of the first PPP projects receiving funding in 2012 focused on pre-breeding of spring barley for disease resistance and adaptation to Nordic conditions. The project was running successfully for three project periods and ended in 2019.
The first period was devoted to obtaining detailed knowledge of the genetic pool available in the Nordic breeding material (Bengtsson et al. 2017a). The activities resulted in several new markers linked to resistance to e.g. the cereal cyst nematode, scald, and powdery mildew (Bengtsson et al. 2017b), and the detection of ideal allele combinations for regional adaptation (Göransson et al. 2019).
The second phase aimed at introducing a larger variability of genes for disease resistance and earliness into adapted material. About 200 diverse genotypes, comprising landraces, breeding lines, and cultivars were evaluated to identify genetic resources for disease resistance and other agronomically important traits. Based on the results from the screening in the first two phases, genotypes were selected and crossed to develop multi-parent advanced generation inter-cross (MAGIC) populations. In total, nine MAGIC populations pyramided for either disease or earliness traits were produced.
The last phase of the spring barley project focused on the genotyping and evaluation of the developed MAGIC progenies using multi-location field trials. The final progenies were evaluated for disease resistance (scald (Hautsalo et al. 2021), mildew (Novakazi et al. 2020), leaf rust, net blotch-net/spot type, and Bipolaris) and heading, maturity, lodging, and height under Nordic field conditions. Additionally, the third phase included high-throughput non-invasive phenotyping of fast seedling growth under controlled conditions in collaboration with IPK, Germany, and with additional funding from an EPPN2020 grant.
Reaching a level of trust and mutual understanding of each partner's needs and limitations is a prerequisite for a successful PPP project. However, the benefits outweigh the efforts. The barley PPP project has provided the Nordic researchers and breeders with improved phenotypic and genetic knowledge as well as MAGIC populations that can be used for breeding disease resistant and regionally adapted spring barley.

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KEYWORDS: MAGIC, Nordic, Pre-breeding, Public-Private Partnership, Spring Barley

BARLEY IN LITHUANIA: A CENTURY LONG BREEDING STORY

Alge Leistrumaite
Institute of Agriculture of Lithuanian Research Centre for Agriculture & Forestry, Instituto ave. 1, Akademija, Kėdainiai distr., LT-58344, Lithuania

alge.leistrumaite@lammc.lt

Barley (Hordeum vulgare L.) is one of the main cereals with a wide range of use. The versatility of barley as a raw material has given it a special place in agriculture and the processing industry. According to the FAO, barley ranks fourth in the world in terms of area and yield. As in the whole of Europe, the area of barley crops in Lithuania tends to decrease (from 352.0 thousand ha in 2000 to 140.8 thousand ha in 2020), but they remain one of the most important among spring cereals

Spring barley varieties have been developed in Lithuania for 100 years. In 1922, the selection of field plants was started in Dotnuva, which is continued today at the Institute of Agriculture of the Lithuanian Research Centre for Agriculture and Forestry

(LAMMC). At the beginning of the work activity of the Plant Breeding Station (1922–1923), efforts were made to preserve the collection of varieties brought from Moscow by prof. D. Rudzinskas and to collect as much initial selection material as possible . During the whole period of plant selection, 27 varieties of spring barley were developed in Dotnuva. They have been registered and cultivated in the country for various periods.Currently, the Lithuanian National List of Plant Varieties and the Common Catalogue of Varieties of Agricultural Plant Species of the EU contain 7 spring barley varieties developed at the Institute of Agriculture (LAMMC).

The Cereals Breeding Department carries out breeding program of spring barley. The main task and purpose of breeders are: development spring barley lines and populations, investigation of their adaptive properties, elements of yield structure and agronomic value, development of promising new varieties, maintenance breeding and seed production. The selection of the most suitable varieties and selection lines of spring barley for organic farming is being carried out.

An important source of initial material is collection of genetic resources. They are constantly supplemented with new barley varieties from European countries and lines with important traits from various Plant Gene Banks. A large (1248 units) collection of barley genetic resources has been accumulated in the Cereal Breeding Department of the LAMMC Institute of Agriculture from 1994. The accumulated rich fund of genetic resources and the available experience allow to continue the development of competitive varieties.

KEYWORDS: Spring barley, Lithuania, breeding

CENTENARY RETROSPECT AND OUTLOOK OF SPRING BARLEY BREEDING AND RESEARCH IN LATVIA

Mara Bleidere
Institute of Agricultural Resources and Economics
mara.bleidere@arei.lv

Co-Author:

Linda Legzdiņa
Institute of Agricultural Resources and Economics

Breeding of spring barley (Hordeum vulgare L.) varieties in Latvia is carried out at the Institute of Agricultural Resources and Economics (AREI) that was established by recent joining of two historical plant breeding institutes (currently named Priekuļi Research Centre and Stende Research Centre). They are located in Vidzeme and Kurzeme regions of Latvia and their rich history goes more than 100 years back. The first barley variety trials were set up simultaneously with the foundation of both institutions - Priekuļi in 1913 and Stende in 1922. The first tested barley diversity included varieties from abroad and landraces collected from local farms consisting of different barley types. Targeted breeding of barley varieties was started in 1924 with the primary goal to develop superior barley varieties for Latvian farmers suitable for regional growing conditions. The first created varieties barley varieties 'Dzintara' and 'Vairoga' characterized with earliness and suitability to unfavorable growth conditions. Further spring barley breeding work over the years in Priekuļi and Stende was done based on specialization (four-row and two-row; feed, malt and food, hulless and covered, organic and conventional) and close cooperation by realizing barley research projects. Keeping in mind the priorities such as yield and its stability, lodging and disease resistance, grain quality criteria for malt, feed and food purposes, and specific criteria for organic farming in total 25 barley varieties have been developed. Currently the Latvian Catalogue of Plant Varieties includes 11 spring barley varieties developed in Latvia. The latest ones are feed barley varieties: high yielding 'Jumara', early ripening 'Saule PR', 'Didzis' possessing yield stability and resistance to leaf diseases. In general Latvian spring barley varieties can be characterized as (1) medium input, with appropriate plant length, straw strength and ripening time suitable for growing also with clover as undersown crop; (2) suitable for organic farming – focused organic breeding program is expanding; (3) usable for feed and food – two varieties 'Irbe' and 'Kornelija' are currently offered in the market. In last five years several research projects were implemented exploring genetic diversity of local barley breeding programs. Agronomic traits significant for adaption to climate change and organic farming were evaluated in multi-environmental field trials. Commercialization strategy for hulless barley variety was developed expanding collaboration with grain processing and food production companies. Agronomic performance and effect of environment were evaluated for genetically diverse barley populations and their improvement techniques are being developed under conditions of organic farming. The long-term co-operation among barley breeders from Baltic countries in evaluating the breeding material should also be noted, interest from Nordic breeders to join have been expanded recently as well.

KEYWORDS: Priekuļi RC, Stende RC, variety characteristics, research projects, co-operation

WHAT IS UNIQUE ABOUT ICELANDIC BARLEY?

Magnus Göransson
Faculty of Agriculture, Agricultural University of Iceland, Reykjavik, Iceland and Department of Plant Science, Norwegian University of Life Sciences, Ås, Norway

magnus.goransson@gmail.com

Co-Authors:

Jón Hallsteinn Hallsson
Faculty of Agriculture, Agricultural University of Iceland, Reykjavik, Iceland

Therése Bengtsson
Department of Plant Breeding, Swedish University of Agricultural Sciences, Lomma, Sweden

Morten Lillemo
Department of Plant Science, Norwegian University of Life Sciences, Ås, Norway

The northwards expansion of barley production requires adaptation to longer days, lower temperatures, and stronger winds during the growing season. In Iceland, the accumulated heat sum in the growth season is around 25% lower than comparable latitudes in Scandinavia, with a mean temperature of around 11.8°C in the warmest month. Here we explore genetic factors underlying the ability of barley to mature in low temperature and long photoperiod.

Early maturing lines with height stability have successfully been developed in Iceland. We have established their performance in multi-environment field trials in six countries ranging from Germany to Iceland. We have also confirmed the early maturity in controlled conditions in growth chambers with contrasting day length and temperature conditions. We used genome wide association studies (GWAS) to identify genetic components behind their early maturity. Re-sequencing of four known earliness genes (PPD-H1, ELF3, FT1, and CEN) identified 12 haplotype combinations. By correlating haplotype diversity with yield data from Icelandic field trials, we identified an allele of PPD-H1 that provided extreme earliness but with a severe yield penalty. An allele of CEN combined earliness with high yield and proved to be beneficial in Icelandic conditions.

The results will aid plant breeders in their work to develop varieties with a shortened time from sowing to maturity under the extreme Icelandic growing conditions and possibly in other arctic or sub-arctic regions. Despite decades of intense breeding efforts relying heavily on the same germplasm, the results show that there still exists considerable variation within the current breeding gene pool, and we identify haplotype diversity for regional adaptation, which could facilitate the expansion of cereal cultivation even further northwards.

KEYWORDS: Icelandic, Nordic, adaptation, CEN, PPD-H1

THE ORIGIN OF THE ICELANDIC BARLEY BREEDING POPULATION

Fluturë Novakazi
Department of Plant Breeding, Swedish University of Agricultural Sciences, Sundsvägen 10, 234 56 Alnarp, Sweden

fluture.novakazi@slu.se

Co-Authors:

Egill Gautason
Center for Quantitative Genetics and Genomics, Aarhus University, Blichers Allé 20, 8830 Tjele

Hrannar Smári Hilmarsson
Agricultural University of Iceland, 311 Hvanneyri Borgarbyggð, Iceland

Iceland is located between 64 and 66°N, with a maritime but arctic climate. Iceland has a relatively long growing season compared to other regions at the same latitude, but summer temperatures are lower in Iceland. Barley was cultivated in Iceland until the 16th century. Although many attempts were made to resume barley cultivation, it was not until the end of the last century that cultivation of barley began to increase. A breeding program for Icelandic barley started in the late 1980s, but due to the long break in barley cultivation, there are no landraces found in Iceland. Therefore, Faroese landraces, other Nordic and Scottish material were used as founders for the breeding population. The breeding program bred for earliness and strong straw and has released eight cultivars so far.

To dissect and investigate the origin of the Icelandic breeding population, we analysed SNP data of 92 barley genotypes consisting of Icelandic breeding lines, Scottish, Faroese and Nordic spring barley genotypes. The panel was genotyped with the iSelect 50k barley SNP array. Monomorphic, unmapped (Morex v3) and SNPs with a call rate < 95% were removed from the analysis, leaving 33 629 SNPs for subsequent analyses. We studied the population structure using model-based-clustering and principle component analysis (PCA). We used model-based clustering to estimate individual ancestry of the 92 genotypes. Linkage disequilibrium (LD) was estimated by calculating the squared allele frequency correlation r2 between marker pairs and chromosome-wise LD decay was estimated by plotting the r2 values against the physical distance with a second-degree smoothed loess curve.

We inferred K = 9 ancestral populations in the data. The Icelandic genotypes had ancestry from Denmark, Finland, Norway, Sweden, Faroe Islands and Scotland. Out of the 65 Icelandic lines, 28 showed admixed ancestry. However, we detected a cluster that was almost exclusively in the Icelandic material. The LD decay ranged between 1.9 Mbp (4H) and 3.4 Mbp (3H). The diversity within the Icelandic genotypes is therefore lower than within the core set (~0.3 Mbp) and lower than within Northern European material (~1 Mbp), but comparable with the Far East material (~2 Mbp). To put the Icelandic genotypes into international perspective, we conducted a PCA including 1000 genotypes from the barley core set in addition to the 95 genotypes based on 38 328 informative SNPs. The first two components explained 9.09% and 7.34%. The Icelandic genotypes clustered with the Northern European genotypes, and showed considerable diversity along the second PC.

The Icelandic barley breeding population is substantially admixed with other Nordic breeding material. Chromosome-wise LD suggests that the Icelandic population is genetically diverse. Genetic diversity has been introduced by crossing with other Nordic material.

KEYWORDS: population genetics, Nordic barley, adaptation, diversity

IDENTIFICATION OF LOCI CONFERRING TOLERANCE TO MANGANESE DEFICIENCY IN SCOTTISH BERE BARLEY LANDRACES

Ronja Wonneberger
Cell and Molecular Sciences, James Hutton Institute, Invergowrie, Dundee, DD2 5DA, Scotland
ronja.wonneberger@hutton.ac.uk

Co-Authors:

Sidsel Birkelund Schmidt, Luke Ramsay, Robbie Waugh, Joanne Russell
Cell and Molecular Sciences, James Hutton Institute, Invergowrie, Dundee, DD2 5DA, Scotland

Timothy George, Lawrie Brown
Ecological Sciences, James Hutton Institute, Invergowrie, Dundee, DD2 5DA, Scotland

Peter Martin, John Wishart
The Agronomy Institute, Orkney College, University of the Highlands and Islands, Orkney, UK

Manganese (Mn), an essential plant nutrient, is an indispensable component of the oxygen-evolving complex in photosystem II and an important co-factor of several enzymes. Mn deficiency severely inhibits the growth and development of cultivars commonly grown in Europe. Soils prone to Mn deficiency are common in many regions of the world. In Scotland, Mn-deficient soils are found in some regions of the Northern and Western islands. Scottish Bere landraces have been grown for hundreds of years on marginal soils on these islands and grow exceptionally well on sandy high pH soils compared to modern cultivars. However, considerable variability in tolerance to Mn deficiency exists among Beres as a result of selection on different soils.

We utilized different mapping strategies to understand and exploit the genetic architecture of Mn deficiency tolerance in Beres. To assess the variation of tolerance to Mn deficiency in Northern European landraces we phenotyped a collection of UK and Nordic landraces including ~30 Beres together with several elite cultivars in a replicated field trial at a Mn-deficient site on Orkney. Using GWAS, QTL for chlorophyll a fluorescence were identified on chromosome 7H and 5H.

We assessed an F2 population derived from a cross between the cultivar KWS Irina and the Mn-efficient Bere Unst from Shetland for chlorophyll fluorescence and leaf Mn concentration on sandy alkaline soil on Orkney. A major QTL spanning a large region of chromosome 6H was identified for chlorophyll a fluorescence and leaf Mn concentration, with the allele conferring tolerance being recessive. In addition, a region on chromosome 2H was associated with leaf Mn content. We intend to phenotype a potentially informative subset of a F6 population from this cross under the same conditions, followed by further genotyping with KASP markers to fine-map the locus.

To facilitate the identification of genes underlying the loci we intend to use a bulking RNAseq approach. Pools of Mn-deficiency tolerant and non-tolerant lines will be grown on soil collected from Orkney, scored non-destructively, RNA extracted, and genes identified that are differentially expressed. We anticipate that genes involved in controlling the acquisition, transport and use of Mn under Mn-deficient conditions will be identified.

A reference-quality genome assembly of Bere Unst will be generated to allow comparative genomic analyses between Beres and elite cultivars.

Together, we illustrate the potential of landraces as a source of traits which are important for the improvement of modern cultivars. Our results indicate that Nordic and especially Bere landraces possess multiple genes that confer tolerance to Mn deficiency and that there is substantial variation in Northern European landraces that can be exploited for breeding. We aim to select progeny of crosses between elite lines and Beres that carry tolerance to Mn deficiency in addition to the desired agronomic traits of the elite parent.

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KEYWORDS: Manganese deficiency, genetic resources, landraces, Bere barley, Scotland