Leibniz-Gemeinschaft

Final report

Title of the project: Graduate School “Yield formation in cereals - overcoming yield-limiting factors”

Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK)
Reference number: SAW-2012-IPK-4
Project period: 2012-2016
Contact partner: Prof. Dr. N. v. Wirén

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Summary

Based on the granted SAW project the IPK established the Graduate School "Yield formation in cereals-overcoming yield-limiting factors" in 2012. For the eight granted PhD students and their projects, this Graduate School developed first an educational program consisting of lectures, seminars, technical workshops as well as scientific and auxiliary courses. It further strengthened the cooperation with the Faculties of Natural Sciences of the Martin-Luther-University Halle-Wittenberg (MLU) as well as with the Leibniz Science Campus Halle “Plant-based bioeconomy”.

The scientific projects funded within the Leibniz Graduate School aimed at identifying genetic and physiological factors that limit the yield potential of barley and wheat. In close cooperation between scientists from IPK Gatersleben and MLU, QTL mapping approaches have been conducted in various barley and wheat populations to determine quantitative trait loci (QTLs) and underlying genes for yield components and/or flower fertility. In addition, transgenic approaches have been used to stabilize hybrids and to regulate the distribution of phytohormones and assimilates in order to improve physiological or morphological traits relevant for yield formation. Six of the eight funded PhD students have successfully completed their PhD and most of the PhD work has been published in peer-reviewed, open-access journals

The structures established with the Leibniz-funded Graduate School have been transformed in 2016 into the IPK Graduate Program. This program is no longer thematically fixed and now hosts all PhD students at the IPK. Structural guideline for a PhD project is the Study Record Book, which contains the initial research proposal, the annual reports and documents the student’s participation in the accompanying scientific and non-scientific coursework besides the central research project. Successful completion of the Study Record Book is awarded with a certificate. Supported by the scientific and administrative coordinators, the PostDoc and the PhD Student Board, the IPK Graduate Program is continuously improved and adapted to the students’ needs. This process is closely supervised and evaluated on a yearly basis by the Scientific Advisory Board of the IPK. Thus, the granted project allowed establishment of a long-term effective, sustainable research and teaching structure, the IPK Graduate Program, in the field of crop plant sciences.

1. Initial questions and objectives of the overall project

The basic concept of this project relied on the establishment of a graduate school at the Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben with the overarching objective to combine at a scientific and structural level the expertise in crop plant research present at the IPK Gatersleben with that present at the Biological and Agricultural Faculties (Faculty of Natural Sciences I and III) of the Martin-Luther University Halle (MLU). Through this integration, it was planned to use the establishment of the Leibniz Graduate School as a starting point to build up a structured graduate program for all PhD students at the IPK that can be maintained afterwards in a sustainable manner, since a structured graduate program for IPK PhD students did not exist before. The common theme chosen for the Leibniz Graduate School was the identification of genetic and physiological factors limiting yield potential in barley and wheat. This topic does not only affirm the enormous relevance of crop plant research for human nutrition but it also matches the mission of the IPK with its engagement in securing food supply and sustainable plant production as well as the thematic focus of the newly established Leibniz-Science Campus Halle “Plant-based Bioeconomy”, allowing further strengthening the affiliation of IPK students to the MLU. In detail, the Leibniz Graduate School pursued two major goals:

Subgoal A: The systematic development of a structured graduate qualification program, whose concept is based on the knowledge-based design of complementary and new approaches (methods) to improve yield formation in cereals. The core of this initial qualification scheme was built first by eight PhD students with interrelated projects, serving as a nucleus for the development of a structured graduate program that is stepwise expanded over all PhD students working at the IPK. In a second phase, the graduate school shall establish as a structural unit of the Leibniz-Science Campus Halle" in which three regional Leibniz Institutes and three faculties of the MLU develop and establish a broad-based educational program for the conceptual promotion of plant-based bioeconomics.

Subgoal B: The elucidation of some genetic and physiological processes that are slowing down or even inhibiting the breeding progress towards grain higher yield in cereals. This goal foresees the establishment of research strategies to increase the yield potential in barley and wheat and was conducted in close cooperation between research groups of the IPK Gatersleben and the MLU. Moreover, these projects were used to support and train the establishment of a structured graduate program. The planned individual projects were the following:

- Project 1: Genome-wide association mapping to genetically dissect yield components in barley (IPK, Genome Diversity group)
- Project 2: Dissection and cloning of genetic factors determining grain yield compo-nents from wild barley introgression lines (MLU, Nat. Fac. III, Plant Breeding)
- Project 3: Whole-genome association genetics for determining floret fertility during the reproductive phase in bread wheat (IPK, Plant Architecture group)
- Project 4: Heterosis fixation in wheat and barley using apomixis candidate genes (IPK, Apomixis group)
- Project 5: Regulation of tiller formation by abscisic acid in barley (IPK, Stress Genomics group)
- Project 6: Stimulation of root growth and grain yield by increased sucrose transport capacity in roots of barley and wheat (IPK, Seed Development group)
- Project 7: Phytohormonal and metabolic regulation of root activity during plant senescence and grain filling (IPK, Molecular Plant Nutrition group)
- Project 8: Regulation of leaf senescence and photosynthetic capacity (MLU, Nat. Fac. I, Plant Physiology)

2. Sub-goal A: Establishment of a Leibniz Graduate School at IPK

2.1. Detailed initial questions and objectives

Before the start of the Leibniz Graduate School, supervision of PhD candidates at the IPK was arranged in a doctoral program that depended mostly on the activities of the direct PhD supervisors at the IPK and the personal engagement of some group leaders that served as teachers or “trouble shooters” and mostly acted at the scientific level, but additionally provided useful hints for the successful completion of a PhD study. Thus, PhD students experienced education and training on an irregular and non-formalized basis. In addition, PhD candidates received support from the Administration, supporting visa issues and other organizational affairs.

To build a framework for the training of graduates in scientific matters and personal skills, IPK had applied for a project to establish a Leibniz Graduate School with funds of the Leibniz SAW program. This project should bring together the already existing structures, and further develop and consolidate a structured educational training program.

With the establishment of such Graduate School, the following advantages were expected: i) With the choice of "Yield formation in cereals" as the research object of the Graduate School, a socially highly relevant topic was placed in the foreground of the scientific strategy of the IPK. ii) Such a structured program promised to increase the number of doctoral, MSc and BSc theses carried out at the IPK. iii) The distance between the IPK Gatersleben and the affiliated universities is relativized by an in-house Graduate Program, thus compensating for the disadvantage of IPK`s remote location. iv) The Graduate School at the IPK fulfilled an initialization function for the sustainable establishment of a structured Graduate Program for all PhD students working at the IPK. The program aims to promote young scientists in their scientific excellence and personal competence. Further aim is to identify individual potentials and to impart knowledge, skills and qualifications that optimally prepare for a successful career. The program is intended to enable doctoral students to pursue their goals, to promote scientific independence and to ensure the quality of scientific work within the framework of doctoral studies.

The educational concept had foreseen to establish first for the eight PhD students supported by the SAW grant an obligatory mentoring, in which the project leader (or PI) together with a university lecturer and a third scientist from IPK form a thesis committee that evaluates on a yearly basis the progress of the PhD project. Written basis is a research proposal serving as an obligatory target agreement between the PhD student and the supervisors, and describes the thesis’ objectives and planned experiments in frame of the dissertation. This proposal also represents the first piece of the Study Record Book listing visited courses and the evaluation results. Furthermore, regular scientific courses were planned, including a lecture series given by scientists of the IPK and MLU offering topical introductions to the theoretical basics of major crop plant research areas and relevant methodological approaches. PhD students should also participate in PhD seminars which were already established at the IPK and the MLU as a venue for students to report on their own projects, or to present current topics from high-impact scientific literature. Further, each PhD student should report at least once a year on the actual state of her/his work in the weekly progress seminar of her/his department and participate in weekly lab meetings to discuss methodological and organizational matters, which also serve as a forum for a journal club to discuss current publications.
To promote methodological skills technical workshops of 1-5 days shalwere offered. In addition, lab rotations were recommended as well as the participation to at least one national and one international conference. To strengthen methodical and personal competences, PhDs were foreseen to participate in regularly held workshops on presentation techniques, project management, communications competences and self-management. The mentoring concept

also considered work-life balance aspects with special attention paid to the promotion of women in science. Before admission to the final PhD examination, entries of the visited educational modules into the Study Record Book were evaluated and granted by a certificate. This educational program required new structures, including the establishment of a Steering Committee, running managerial issues of the Graduate School as well as of a PhD Student Board, organizing a yearly Plant Science Student Conference.

Since the beginning of the Leibniz Graduate School in 2012, initially established organizational structures underwent a yearly review by the Scientific Advisory Board of the IPK. Following their advices, and based on regular meetings and podium discussions with the PhD students or the PhD Student Board, the concept of the Graduate School underwent several modifications, which cannot be listed here in detail. Thus, the following report on the instruments of the Graduate School places the focus on the current state of the educational items. Here, it is important to note that with the end of the SAW project on the establishment of a Leibniz Graduate School, which had a thematic focus on "Yield formation in cereals - overcoming yield-limiting factors", the IPK decided to change the title of the Graduate School to the IPK Graduate Program (https://www.ipk-gatersleben.de/en/ipk-graduate-program/). This name change facilitated integration of finally all PhD students at the IPK as well as their acceptance to become part of this program, irrespective of their research topic. Meanwhile, the IPK Graduate Program has become obligatory for all doctoral students at IPK. Exceptions arise only from enrollment requirements at individual universities or funding programs.

In a wider frame, the IPK Graduate Program now fits into a broad range of career development opportunities and instruments at IPK, which offer doctoral students and young scientists a broad spectrum of professional and personal supervision and support in an excellent research environment. In particular, the PhD Student Board (https://www.ipk-gatersleben.de/en/student-board/) and the PostDoc Board (https://www.ipk-gatersleben.de/en/postdoc-board/; both are self-organized units) and the Equal Opportunities Office must be named here.

Another important point to consider is that the PhD degree is awarded by the universities to which the IPK is affiliated. This is in first instance the MLU Halle, but there are also long-standing cooperations with the universities of Kiel, Greifswald and most recently Göttingen. Formal requirements for the doctorate (according to the requirements and prerequisites of the respective valid doctoral regulations of the respective faculty) are rules and deadlines for the start of the doctoral examination procedure and the submission of the dissertation to the respective university. Thus, the IPK cannot decide on the admission of a PhD student to the examination at a university faculty. Therefore, the IPK decided to award a certificate to each PhD student who has successfully completed the Graduate School or IPK Graduate Program.

2.2. Development of the work carried out / presentation of the results achieved / possible application perspectives and conceivable follow-up projects

The IPK started in February 2012 with the acquisition of suitable qualified students in a recruitment procedure, which was followed by a selection process. The selection procedure was carried out with the participation of the scientific coordinator and spokesperson of the Graduate School, Prof. Dr. N. von Wirén, the administrative coordinator Dr. B. Leps, and the appointed steering committee, consisting of the deputy spokesperson Prof. A. Graner (IPK), Prof. K. Pillen (MLU), Prof. K. Humbeck (MLU) and Dr. Tim Sharbel (IPK) as well as the other project managers of the eight subprojects.

The eight vacant positions were advertised on a large number of national and international platforms and in digital student networks. The selection procedure was divided into two

phases. In the first phase, the steering committee and the respective project managers preselected suitable candidates on the basis of the extensive written application documents submitted by the individual candidates. In the second phase, 2 to 3 candidates for each project were invited to the IPK in Gatersleben for a 2-days personal introduction and interview. The on-site visit in June 2012 was introduced by a lecture of the scientific coordinator about the objectives, the structure and the program of the graduate school and followed by lectures of the respective project leaders about the subproject contents. Then, the invited candidates presented their previous scientific work. Subsequent personal interviews allowed the project leaders to gain an impression of the specific professional suitability and motivation of the candidates. The steering committee and the project leaders then selected eight candidates from the 20 invited candidates, who began their PhD work at IPK Gatersleben and MLU in Halle starting in October or November 2012 (see Part B).

A further focus in 2012 was the development and implementation of a concept for structured graduate training and qualification (https://www.ipk-gatersleben.de/en/ipk-graduate-program/), as well as the establishment and further development of the instruments and courses to be offered. To this end, a new program consisting of lectures, scientific and technical as well as accompanying non-scientific courses was set up and coordinated with the activities of the doctoral program already existing at the IPK as well as with other qualification offers, in particular colloquia and seminars (Figure 1).

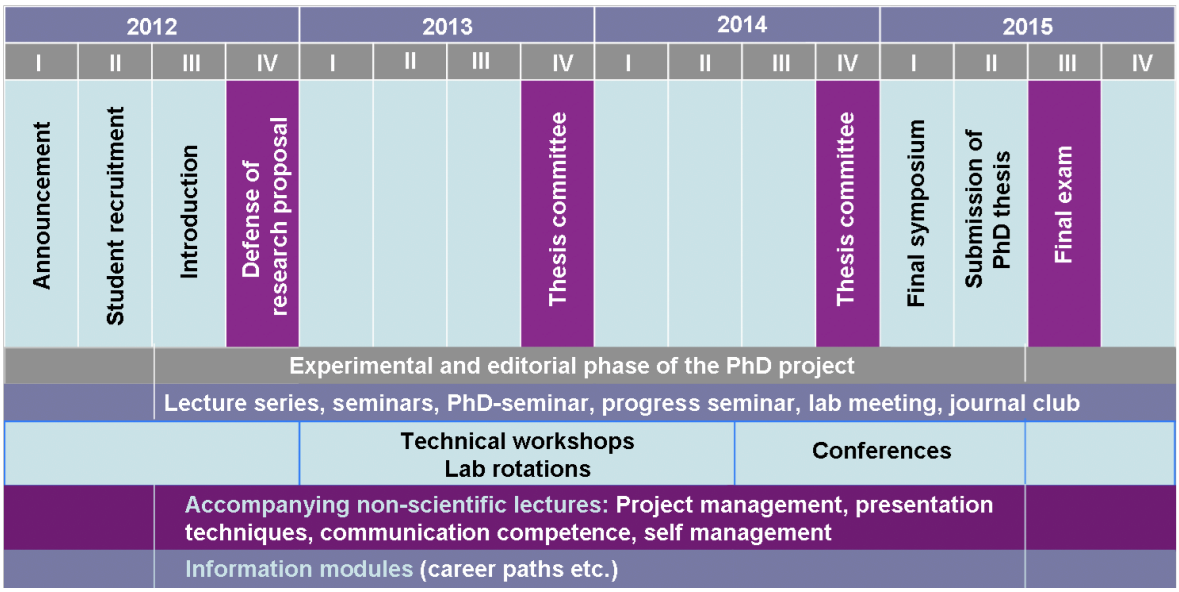

Figure 1. Operation chart for the program realized within the Leibniz Graduate School "Yield formation in cereals". A PhD thesis is divided into three phases, starting with the setup and defense of the Research Proposal, marked by the submission and evaluation of the Annual Reports by the Thesis Committee and completed by the PhD examination. During all three phases PhD students conduct their experimental work and participate in accompanying scientific coursework, technical workshops and "soft skill" courses to strengthen their personal competences.

Within the framework of the IPK Graduate Program, new instruments had to be developed to ensure the desired structured qualification of doctoral students. The most important ones are the following:

Study Record Book

The established Graduate School is characterized by transparency and the determination of mutual responsibilities of the PhD student and the supervisor(s) for all phases of qualification up to achieving the doctoral degree. Therefore, the coordinators developed the Study Record Book (http://www.ipk-gatersleben.de/promotion/graduate-school/programm/) in order to de-

fine the overarching goals described in the individual research proposals and to create a basis for structuring the scientific and educational framework. From the perspective of the PhD students, the Study Record Book serves the PhD students primarily for the structured and chronological documentation of the project goals and progress (Research Proposal, Annual Report) as well as the proof of participation in different lectures, technical courses and workshops.

In particular the Research Proposal and the Annual Reports became essential instruments within the framework of the qualification. The Research Proposal serves as a binding agreement between a PhD student and his supervisors, including the mentor, with regard to the content and timing of the work to be undertaken during the doctoral thesis as well as the implementation of the planned practical work. It contains an introduction to the topic including some literature review, describes the main objectives of the doctoral thesis and elaborates on the planned experimental approaches for the current or, in case of the Annual Report, on the next phase of the doctoral project. The Research Proposal/Annual Report should be discussed at least once a year with the Thesis Committee on the basis of a short written report on the results achieved. The annual meeting with the supervisor and mentor then allows to confirm or formulate novel intermediate goals and, if necessary, to adjust the time required to fulfill the overall thesis objectives. This procedure has been proven crucial to enable successful completion of the doctoral studies and reception of the doctoral degree promptly after the end of the respective funding period.

The Study Record Book is checked regularly by the administrative coordinator to ensure timely submission of the dissertation at the end of the respective funding period and prompt fixation of the oral exam. Initially, this funding period was expected to terminate after three years. However, in reality it turned out that most PhD projects cannot deliver satisfying scientific novelties within this duration, and most PhD students require in average four years for their scientific and personal qualification. In such cases, the IPK ensures continued financial support of the PhD student through budgets allocated to the group leaders that allow them to continue employment of the PhD student.

Thesis Committee and mentoring

Another instrument that was introduced is the Thesis Committee, consisting of the project leader, another group leader or university lecturer and a mentor. The Thesis Committee evaluates the progress of the project on the basis of the Research Proposal or the Annual Report. PhD students are required to discuss and document the progress of their work with their Thesis Committee members at least once a year within a period of 6 months after starting their doctoral thesis. The short protocol of at least 1-2 pages including the Research Proposal and the Annual Reports are essential parts of the Study Record Book.

More recently, the Graduate Program has been striving for a stronger link between the research projects running in the institute and in the Graduate Program. On the one hand, this has been realized by the definition of five Research Themes of the IPK (https://www.ipk-gatersleben.de/en/research/research-themes/), which allow each PhD project to be assigned to one of them. On the other hand, this is realized through mentoring. All doctoral students are asked to find a mentor and discuss the progress of their research project with him or her. The aim is to support the progress of the individual projects by means of continuous monitoring in order to work purposefully towards a successful conclusion in due time and to solve promptly any difficulties that may arise.

Scientific lectures

This part of the program is based on lectures given by IPK scientists as well as external scientists and university teachers, which take place alternately with the PhD seminars conduct-

ed by the PhD students. A prerequisite for the establishment of a comprehensive and structured course program was and is the participation of senior and experienced scientists, employed at the IPK, who predominantly offer subject-specific lectures. The organisation, preparation and follow-up of the courses is carried out in close coordination with the respective scientists and the coordination of the Graduate School. Initially these lectures were offered in a kind of ring presentation and served as an introduction to the theoretical basics and current scientific research areas at the IPK and other research institutions.
Recently the lecture series was replaced by so-called Departmental Days. These are held twice a year. At the Departmental Day the research groups of a department give a more general overview about their research topics. Thus, the PhD students get a general overview and new insights into the research within the different disciplines of the institute and learn to connect specific research questions with appropriate approaches, not necessarily linked with their own work.

Scientific-technical workshops

Scientific (technical) courses: During a 1- to 5-day course, PhD students are familiarized with new methods such as genetic, biochemical and/or cell biological approaches to promote methodological knowledge and background knowledge in various areas of plant biotechnology. Each year several courses on different topics are offered (see Section 7).

Auxiliary non-scientific workshops

The following workshops are offered as internal workshops to improve the professional qualifications of doctoral students: Project management (project conception and structure, cost statements, reimbursement, international project management, cooperation etc.), presentation techniques (scientific writing, lectures, posters, rhetorics, teaching rules etc.), communication skills (speaking, work organisation, reflection skills, intercultural conflict management etc.) and self-management (time management, work organisation, media competence, "learn to learn", career paths in science or industry, application procedures and interviews etc.). General information modules include: Good Scientific Practice, Scientific Writing and Publishing, etc. (see Section 7). PhD students can also register for the courses offered by InGra at the MLU.

Based on the experience collected during these workshops, the feedback by the workshop participants and the experience made during the support of the doctoral students over the past four years, the IPK is currently establishing a non-scientific training program of the doctoral students through interactive mentoring and - as far as required - individual coaching, especially with regard to the promotion of women in science.

Seminars

PhD seminars: These seminars offer the opportunity for PhD students to report on one's own field of research as well as on a "hot topic" in front of other PhD students. This seminar is organized and supervised by the PhD students themselves. The seminars will be supervised by an experienced scientist, who acts as a mentor and helps structuring the discussion.

Progress seminars: PhD students must present the results of their own scientific work at least once a calendar year in the progress seminar of their department. The seminars are organized and announced by the individual departments. The attendance to each progress seminar in the department is obligatory.

Conferences/ poster presentations, talks

The Graduate Program aims at the visibility of the overall project, its goals and intermediate results during the research phase. Therefore each PhD student should participate in the annual Plant Science Student Conference (https://www.ipk-gatersleben.de/en/student-board/science-and-education/) , which is organized by the PhD Student Board of IPK, in cooperation with PhD students of the MLU and the IPB Halle. In addition, each PhD student takes part in at least one international conference to present her/his own data. Participation in meetings and conferences allows practicing the exchange of scientific information, knowledge and experience and the establishment of new national and international contacts as well as new scientific networks within the scientific community. Each PhD student must participate actively and at least twice in meetings and exchange ideas with international speakers.

Transparency/ quality assurance

The Graduate School "Yield-limiting factors" (https://www.ipk-gatersleben.de/en/phd-students/leibniz-graduate-school/) and the actual IPK Graduate Program (https://www.ipk-gatersleben.de/en/ipk-graduate-program/) have presented and are still presenting their activities transparently on the IPK website as well as on the intranet. This concerns the objectives, the projects and the course and lecture program as well as other events such as guest lectures, excursions, conferences, external workshops and summer schools.

Moreover, the use of feedback forms for the lectures, courses and workshops offered at the Graduate Program was established, which have to be completed by the participants for the purpose of quality assurance of the program. In this way, the Graduate Program can be continuously improved and further adapted to the needs of the PhD students. Here, it is important to note that the PhD Student Board has undergone a remarkable development. Starting initially from the role of a students' union that critically commented on the Graduate Program and organized the Plant Science Student Conference, the PhD Student Board is now directly involved in the organization of the contents of the educational program. It runs an own financial budget e.g. for the organization of the yearly conference, takes over mentoring responsibilities for newcomers, has established the Beagle award for exceptional PhD students and organizes social activities. This active role in structuring the PhD education has made the PhD Student Board becoming a major player in the whole IPK Graduate Program.

Conclusion

The project granted by the SAW led to the establishment of a Leibniz-Graduate School "Yield formation in cereals – overcoming yield-limiting factors". From the recruitment of doctoral students in 2012 it ran until 2016 before it was transformed into the IPK Graduate Program. This program has become obligatory for all PhD Students at the IPK and is awarded with a certificate after successful completion of a given number of attended lectures and courses besides the central research project. The Graduate Program has been and will be continuously expanded, supplemented, improved and adapted to the students` needs and requirements of the scientific system(s). The lectures, courses and workshops are offered throughout the calendar year and are repeated on a regular basis so that a qualitative education and further training of the doctoral students can be facilitated and guaranteed throughout the entire period of a doctoral thesis irrespective of its starting date. This process is closely supervised and evaluated on a yearly basis by the Scientific Advisory Board of the IPK.

3. Sub-goal B: Scientific questions Graduate School "Yield formation in cereals – overcoming yield-limiting factors"

3.1. Overall objectives and added value

Due to its diploid genome barley is amenable to a wide range of gene identification and genomic approaches, while research in hexaploid wheat requires more time and resources. Since barley qualifies as a model plant for Triticeae research and knowledge on yield formation can be transferred between the two species, the GS employs approaches in either one or both of these species. The projects 1-3 directly interrogated the genome for genes or gene loci determining individual yield components, while projects 4-6 employed transgenic approaches to enhance and stabilize yield potential. Projects 7-8 utilized physiological means to extend the lifespan of source organs for prolonged nutrient and assimilate delivery to grains.

The parallel establishment of genetic, genomic, transgene and physiological approaches provided an ideal opportunity for timely exchange of genetic material and methodological tools which accelerated research progress. This synergy led to the rapid identification of contrasting genetic lines for characterization of underlying yield-forming processes, an early verification of those physiological processes which are subject to genotypic variability and might be affected by mapped QTLs. This focused and interactive research concept provided a great opportunity for the IPK to advance a scientific core area with high international visibility and impact. The interaction of these projects with ongoing projects in the fields of seed biology, physiology and yield formation positioned the Leibniz-Graduate School at the heart of a scientific network that still provides plenty of opportunities for collaborations among PhD students.

The main objectives, the development of work, findings and publications are outlined below.

3.2. Project 1: Genome-wide association mapping to genetically dissect yield components in barley (IPK, Genome Diversity group)

Objectives

Yield improvement in barley involves the identification and mobilization of new alleles from locally adapted materials into elite germplasm by breeding. This project aimed at the map-based identification of genomic loci and underlying genes determining yield components in barley lines representing a world-wide cross-section of the genomic variability of cultivated barley.

Development of the work carried out and perspectives

A collection of 261 two-rowed spring barley landraces of diverse origin were grown for two years at IPK Gatersleben. In parallel, these lines were genotyped by using the iSelect 9K chip. Fourteen agronomic traits were measured to identify novel yield-related quantitative trait loci (QTLs) in a genome-wide association study (GWAS). For all traits, a high phenotypic variance was observed across both years with heritability estimates ranging between 0.74-0.95. In total, 331 marker-trait associations (MTA) were identified for all 14 traits and grouped into 79 QTLs spanning the entire barley genome. Most identified QTLs for individual yield-related traits including flowering time, plant height and thousand grain weight coincided with genomic regions of known barley genes, like with the photoperiodic gene Ppd-H1 (2H), earliness per se (CENTRORADIALIS) (2H), or the *waxy* (7H) locus.

An interesting discovery was the identification of a second QTL for barley hull adherence on chromosome 3H, which was specific for naked barley originating from Europe and Asia. The observation that this association is specific to naked barley from Eurasia reflected the allelic diversity at this genomic region in the whole population. To date, only the Nud gene on chromosome 7H has been identified, cloned and functionally characterized with regard to barley hull adherence. Within the genomic region of the 3H QTL is Eibi1, encoding an ABC transporter involved in cutin biosynthesis, which has been reported to be regulated by the Nud and Epb1, a major cysteine proteinase responsible for the degradation of endosperm storage proteins in barley. However, the direct involvement of Eibi1 in hull adherence could not be elucidated. Though there is no report on the involvement of either Eibi1 or Epb1 in hull adherence, these two genes are potential candidates and need to be investigated in more detail.

The results highlight the importance of the current panel of barley landraces for novel QTL identification and could serve as a starting point of marker-assisted selection (MAS) for yield improvements.

Publications

Wabila, Celestine (2018). A genome-wide association study to genetically dissect yield related traits in a diverse collection of spring barley landraces. (PhD Thesis) Halle/S., Martin-Luther-Universität Halle-Wittenberg, Institut für Agrar- und Ernährungswissenschaften der Naturwissenschaftlichen Fakultät III, 142 pp.

3.3. Project 2: Dissection and cloning of genetic factors determining grain yield components from wild barley introgression lines (MLU, Nat. Fac. III, Plant Breeding)

Objectives

To identify novel genes of the wild barley (*Hordeum vulgare* ssp *spontaneum*) gene pool determining grain yield potential, we have introgressed wild barley germplasm into elite barley (*H. vulgare*) cultivars. Using genome-wide association studies (GWAS), multiple QTLs controlling grain yield and grain weight were mapped in introgression lines (Schmalenbach et al. 2009) and in a nested association mapping population (Maurer et al. 2016, Sharma et al. 2018). The selection of free-threshing spikes was a major domestication trait selected during the transition from wild to cultivated barley. The threshability phenotype in barley is controlled by *Thresh-1* (Schmalenbach et al. 2011). The dominant cultivar allele causes an easy to thresh phenotype, whereas the recessive wild barley allele produces grains, which are still attached to rachis and awns despite of mechanical threshing. In project 2, we aimed to fine-map and ultimately clone the *Thresh-1* locus conferring low threshability and reduced yield in wild barley.

Development of the work carried out and perspectives

The nested association mapping population HEB-25 was used to ultra fine-map the *Thresh-1* locus on chromosome arm 1HL (Pencs et al., in prep.). We further reduced the extent of the *Thresh-1* locus using a whole-exome capture approach comparing easy and difficult to thresh sister NAM lines and assigned the locus to a single Morex contig of the barley physical map. Subsequently, four candidate genes for *Thresh-1,* separated by approximately 0.4 Mbp, were identified after in-depth transcript profiling based on RNAseq analysis. Furthermore, we detected a nucleotide mutation in one of these genes, which is consistent with the dominance of the easy threshable cultivated allele over the recessive difficult to thresh allele. Currently, we are producing targeted knock out mutants through genome editing to prove, which of the four candidate genes are responsible to cause the *Thresh-1* phenotype (collaboration with Drs. J. Kumlehn and G. Hensel, IPK Gatersleben).

The presented work laid the foundation to explain how mutations were selected approximately 10,000 years ago during domestication in order to adapt ancient cereals to the requirements of modern agriculture. Currently, a master fellowship, provided by KWS SE, Einbeck, supports the genome editing approach in Gatersleben. As soon as the gene/s are validated, a DFG follow-up study will be filed to further study the molecular regulation of threshability and the genetic variation of *Thresh-1* in barley and related cereal species.

Publications

Pencs S. High-resolution mapping of a candidate gene for *thresh-1*, a domestication gene causing a difficult to thresh phenotype in wild barley germplasm. Martin-Luther-Universität Halle-Wittenberg, Institut für Agrar- und Ernährungswissenschaften der Naturwissenschaftlichen Fakultät III, *submission of PhD thesis pending*

Pencs S., Mascher M., Stein N., Himmelbach A., Schmutzer T., Bull F., Große I., Fincher G.B., Burton R., Pillen K. High-resolution mapping and identification of a candidate gene for *thresh-1*, a domestication gene causing a difficult to thresh phenotype in wild barley germplasm, *in preparation*

3.4. Projekt 3: Whole-genome association genetics for determining floret fertility during the reproductive phase in bread wheat (IPK, Plant Architecture group)

Objectives

Although wheat produces florets indeterminately, early degeneration of the florets has been a major challenge for increasing yield directly by increasing floret fertility. Furthermore, there is no clear understanding of the cause for floret abortion (degeneration) in wheat. Hence, increasing grain yield by increasing fertile floret is not an easy task. Therefore, other options need to be sought. One of these is increasing spikelet number per spike.

Development of the work carried out

Wheat inflorescence, also known as the spike, bears a determinate number of spikelets arranged in a distichous order on the main axis of the spike. Wheat strain known as 'Miracle wheat' or the branched head wheat shows a non-canonical spike form where secondary spikelets and/or small-sized mini-spikes appear from the central axis of the spike. Besides the previously identified gene underlying the 'Miracle wheat' or the branched head (bht) locus, the current study identified two additional QTL on chrs 2BS and 1AS controlling 'Mir-acle wheat' phenotype in tetraploid wheat. Further analyses showed that the QTL on Chr. 2BS (QSS.ipk-2BS) is found to be the homoeo-locus of the 'Miracle wheat' QTL on chr.2AS (QSS.ipk-2AS). Furthermore, 'Miracle wheat' Near Isogenic Lines carrying the 'Miracle wheat' allele (bh-A1) were developed as unique genetic resources to further studying the genetic basis of wheat spike development as well as the source-sink relationship in wheat.
Source-sink manipulation through de-tillering of FL-bh-NILs plants at BC3F3 lead to about 65% improvement in supernumerary spikelet fertility after de-tillering, suggesting that reduced spikelet fertility during spike- branching is partly connected with assimilate availability and distribution. The differential spikelet fertility between primary and secondary spikelets, both of which are connected to similar assimilate source through the shared node, suggests that there might be a limited, and yet unknown assimilate allocation criteria in the spikelets and florets of wheat. Nevertheless, assimilate allocation in wheat spikelet/floret might follow the 'Aorta Model' where the main vascular bundle (assimilate source) bifur-cates following Murray's law at each node point of each spikelet and floret. Hence, source capacity and size of the vascular bundle or allocation criteria might be the major factors controlling the amount of assimilate allocated to specific sink organ affecting spikelet and floret fertility in wheat.
Studying source-sink relation in wheat is a key factor for unlocking grain yield limitations in wheat. However, available information concerning the source-sink relationship in wheat is largely inconsistent as to whether yield limitation in wheat is source or sink driven. Hence, the developed FL-bh-NILs provide further opportunity to understand and genetically dissect source-sink relationship in wheat. Furthermore, combing *bh* allele(s) with other loci may pave a way for gene pyramiding towards creating the ideal wheat plant architecture.

Publications

Wolde G.M. (2017). Exploring modified durum wheat (*Triticum durum* Desf.) plant architecture. Martin-Luther University Halle-Wittenberg and Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, 189 pp.

Wolde, G.M., Schnurbusch T. (2019) Inferring vascular architecture of the wheat spikelet based on resource allocation in the branched headt (bht-A1) near-isogenic lines, *in review*

Wolde, G.M., Trautewig C., Mascher M., Schnurbusch T. (2019) Genetic insights into morphometric inflorescence traits of wheat. Theor. Appl. Genet., online

Wolde, G.M., Mascher M., Schnurbusch T. (2019) Genetic modification of spikelet arrangement in wheat increases grain number without significantly affecting grain weight. Mol. Genet. Genomics, online

3.5. Project 4: Heterosis fixation in wheat and barley using apomixis candidate genes (IPK, Apomixis group)

Objectives

Poa pratensis, an important forage and turf grass characterized by variable ploidy, a huge genome and versatile reproduction mode, ranging from obligate sexual to facultative apomictic to obligate apomictic, is a useful model for testing these evolutionary processes. The spread of apomixes in natural populations leads to mixed populations with varying levels of apomixes penetrance. The goal of the project was to perform a comparative gene expression study on microdissected live ovules collected from sexual and apomictic individuals of *Poa pratensis* to test (i) if the genetic variations in Poa accessions reflects multiple apomixes origins, (ii) if polyploidisation is correlated with apomictic penetrance, and (iii) elevated genetic variation can be used as a tool for identifying conserved apomictic factors in ovule-specified transcriptome analyses.

Development of the work carried out

The experimental work started with an overview of the genetic diversity of available accessions and populations on a wide geographical scale as the analysis required a large number of biological replicates of each reproductive form. This was crucial for the identification of differences among regions and among agricultural accessions and wild type populations as well as to increase the probability of differentiating between i) genotype-specific variations in gene expression and ii) conserved differences between the sexual and apomictic pathway. This was followed by the determination of the reproductive pathways in chosen individuals to classify highly apomictic and sexual genotypes. We used genome wide expression profiling microarrays to identify apomictic factors that could be potential candidates for a breeding program.

133 accessions from 29 different countries have been collected. Due to the highly variable ploidy and genetic diversity the functional approach couldn`t be achieved. Therefore, the focus was shifted towards a more evolutionary approach, especially on the coherence of polyploidy, genetic diversity and hybridization with apomixes penetrance in *Poa pratensis*. In addition to the 133 accessions of *Poa pratensis*, an outgroup of approx. 33 Poa spec. was established for phylogenetic analysis of the genus.

As methodological approach we used among others caryotyping of root tips of one individual per accession; microsatellite analysis, cpDNA sequencing using ndhF and trnL-trnT primers as well as Flow Cytometric Seed Screening (FCSS) to quantify reproductive pathways.

A structured analysis of microsatellite data identified three different groups within the data set, but none of the tested biological parameters reflected the observed clusters. The distribution of pairwise genetic distances reflected that for a sexual, rather than asexual population. This lead to the hypothesis that even limited amounts of sexual reproduction, in combination with ongoing hybridization, can obscure patters of asexuality in Poa pratensis.

Publications

None. PhD candidate discontinued PhD project at later stage, partly due to the move of the group to Canada.

3.6. Project 5: Regulation of tiller formation by abscisic acid in barley (IPK, Stress Genomics group)

Objectives

Strigolactones (SLs) represent a class of plant hormones involved in inhibiting shoot branching and in promoting abiotic stress responses. Evidence has shown that SLs and abscisic acid (ABA) biosynthesis pathways are functionally connected. However, little is known about the mechanism underlying the cross-talk between ABA and SL and the relevance of this cross-talk for shoot branching. In a RNAi approach for the silencing of a cytochrome P450 gene involved in homeostasis of the stress hormone abscisic acid (ABA) in barley, two independent transgenic lines have been generated that show lower degradation of ABA. Interestingly, these two lines generate up to 20% more tillers in the generative phase, leading to a strong increase in yield in greenhouse trials. This project thus aimed at investigating whether there is a cross-talk between ABA and SLs in the regulation of tiller formation during the transition from vegetative to generative growth.

Development of the work carried out

As a prerequisite for expression analysis of genes involved in SL biosynthesis, genomic sequences of the barley orthologs for D27, CCD7, CCD8 and MAX1 were identified. D27 and MAX1 were functionally characterized by heterologous expression in corresponding Arabidopsis mutants, in which they partially rescued their shoot branching phenotypes. Furthermore, virus-induced gene silencing was employed to show that HvD27-silenced, HvCCD7-silenced and HvCCD8-silenced plants not only showed increased tillering but also produced more additional spikelets and influenced the spikelet fertility.

To investigate cross-talk between ABA and SLs, transgenic barley lines were employed, in which the gene HvABA 8'-hydroxylase was silenced. This gene encodes an enzyme catalyzing the hydrolysis and thus the catabolism of ABA. The two corresponding transgenic lines LOHi236 and 272 continued tillering during the reproductive phase, resulting in enhanced tiller numbers. LC-MS/MS analysis of root and stem base tissues confirmed higher ABA levels in the two transgenic lines due to the downregulation of the two isoforms HvABA 8'-hydroxylase 1 and 3. In addition, concentrations of the SL 5-deoxystrigol, which could only be quantified in root exudates, were lower. Gene expression analysis further confirmed that the accumulation of ABA in the transgenic lines led to a repression of SL biosynthesis genes, in particular of HvCCD7 and HvCCD8, probably as a result of a feed-back regulation by elevated ABA levels. However, the de-regulation of gene expression was only seen at 14 weeks after germination, i.e. when plants had entered booting stage, which was most likely due to the developmental regulation of the promoter activity driving the transgene. Taken together, these results indicate that enhanced levels of endogenous ABA can decrease SL production, which triggers enhanced tillering. Thereby, this study provides first genetic evidence for a direct cross-talk between these two phytohormone classes that takes place at the level of their biosynthesis.

Publications

Wang, H (2017). Genetic manipulation of the cross-talk between abscisic acid and strigolactones and their biosynthetic link during late tillering in barley. (PhD Thesis) Halle/S., Martin-Luther-Universität Halle-Wittenberg, Naturwissenschaftliche Fakultät I Biowissenschaften 114 pp.

Wang H., Chen W., Eggert K., Charnikhova T., Bouwmeester H., Schweizer P., Hajirezaei M.R., Seiler C., Sreenivasulu N., von Wirén N., Kuhlmann M. (2018) Abscisic acid influences tillering by modulation of strigolactones in barley. J. Exp. Bot. 69: 3883-3898.

3.7. Project 6: Stimulation of root growth and grain yield by increased sucrose transport capacity in roots of barley and wheat (IPK, Seed Development group)

Objectives

Root development during the vegetative growth phase of cereal growth improves water and nutrient uptake and stabilizes yield formation under adverse growth conditions. Transgenic wheat lines were analyzed ectopically expressing the sucrose transporter HvSUT1 under control of wheat 1Ax1promotor. The aim was (i) to evaluate the potential of improved amino acid uptake capacity for grain yield, (ii) to characterize seedling root phenotype, (iii) to evaluate the influence of *VfAAP1* expression on grain development at the transcript level. Grain yield and properties were determined from plants of growth chambers and greenhouses. Using barley lines expressing hordein B1-promoter:GFP, promoter activity was characterized. Comparative transcriptome analyses of transgenic and wild-type grains were performed to identify candidate genes underlying improved root growth and yield formation.

Development of the work carried out

The present study analyzed the potential of improving amino acid uptake capacity on developing grains for yield formation in wheat. Grain yield per plot was increased due to higher gran weight. The 1Ax1 promotor was active in developing endosperm, maternal XAP grain tissues and in seedling roots. XAP lines displayed altered seedling root architecture with more seminal roots per seedling, shorter seminal roots, higher root dry mass, and more root branching points. The *VfAAP1* transgene was differently expressed in distinct root parts of XAP seedlings with highest levels in the cell maturity zone. Expression was lower in the cell elongation zone. Transcriptional changes in XAP and control grains were evaluated by transcript profiling Results show general activation of transport processes in the young pericarp and upregulation of cell division- and elongation-related genes in endosperm at the transition phase. Transport- and storage related genes were upregulated in XAP pericarp and endosperm. Cell wall related genes were preferentially upregulated in XAP pericarp. Genes related to cell division including cyclins and CDKs are upregulated in XAP endosperm suggesting stimulated cell division and cell growth. Genes related to protein storage synthesis, specifically of the prolamin class, were upregulated in XAP endosperm. Expression of metal transporter genes including Zn and Fe transporters were also upregulated in XAP endosperm. Correspondingly, genes related to the biosynthesis of nitrogen chelates like nicotianamine synthase are upregulated in the XAP endosperm. Consistent with this result, significant levels of Zn and Fe occurred in mature XAP grains.

Grain protein content is an important trait in wheat but the negative correlation of grain protein content and grain yield is a potential challenge for breeders. Genetic and physiological understanding of trade-offs among yield traits could be fundamental to increase grain yield and quality. The data suggest that *VfAAP1* expression increases amino acid uptake into developing grains resulting in formation of higher grain weight and stimulated storage protein synthesis. Thus, increased transcriptional activity of sink located amino acid transporters positively affects sink strength and that N assimilate uploading into developing grains might be a limiting step for sink development and therefore for yield improvement in wheat.

Publications

Abebaw Y. (2017). Ectopic expression of a *Vicia faba* amino acid permease (VfAAP1) improves grain yield and stimulates seedling root growth in wheat (*Triticum aestivum*). (PhD Thesis) Halle/S., Martin-Luther-Universität Halle-Wittenberg, Naturwissenschaftliche Fakultät I Biowissenschaften 127 pp.

3.8. Project 7: Phytohormonal and metabolic regulation of root activity during plant senescence and grain filling (IPK, Molecular Plant Nutrition group)

Objectives

Plant roots serve important functions, such as water and nutrient uptake. These functions are altered during the lifespan of a root, thus determined by plant or root aging. Despite the importance of root aging processes in plant performance, the mechanisms determining and regulating root senescence have remained unknown. Thus, the aim of this project was to elucidate root aging or root senescence processes at the morphological, physiological and molecular level in seminal roots of hydroponically-grown barley plants.

Development of the work carried out

Over a period of 53 days, root senescence processes were investigated by microscopy for structural modifications, by nutrient uptake studies using stable isotopes, by phytohormone analyses and by transcriptome studies It was found that arrested root elongation, lysis of the root cortex, and decreases in nitrate uptake rate as well as in auxin and cytokinin concentrations coincided at the same time point, i.e. 39 days after germination (dag). At this time point, root abscisic acid (ABA) levels peaked, which was confirmed by enhanced transcript levels of ABA biosynthesis genes, suggesting that ABA may act as a trigger for root aging-relates processes. Transcriptome profiling in apical and basal root tissues revealed a continuous upregulation of several NAC-, WRKY- and AP2 type transcription factors from 32 or dag on. At 46 and 53 dag, genes involved in proteolysis and oxidative stress responses became upregulated. This work represents the first comprehensive study on root senescence. It identified promising candidates indicative for root senescence at the morphological (degradation of cortical cells), physiological (tryptamine, serotonin) and at the molecular level (ROS-related genes and transcription factors) that appear to act in a temporally coordinated manner. Based on these observations, it is concluded that the degenerative process in aging seminal roots underlie a genetically determined program that can be assigned to root senescence.
The present work laid the basis for a follow-up project directed towards the identification of the regulatory role of some of the identified transcription factors in root senescence. In a transgenic approach, two of the above-identified transcription factors are knocked out or overexpressed only in roots. This project is funded by the state Saxony-Anhalt in frame of the graduate school Agripoly at the MLU Halle.

Publications

Liu, Zhaojun (2018) Morphological, physiological and molecular characterization of root senescence in barley. (PhD Thesis) Halle/S., Martin-Luther-Universität Halle-Wittenberg, Naturwissenschaftliche Fakultät I Biowissenschaften 135 pp.

Liu, Z., Hartmann A., Eggert K., Hajirezaei M.R., von Wirén N. (2019) A chronological sequence of senescence processes in the seminal root system of barley; *submitted*.

Liu, Z., Hartmann A., Hajirezaei M.R., Carpentier S., von Wirén N. Proteome profiling reveals the expression of specific proteins in seminal and nodal roots of barley; *in preparation*.

3.9. Project 8: Regulation of leaf senescence and photosynthetic capacity (Plant Physiology, MLU, Nat. Fac. I)

Objectives

Leaf senescence is a developmental process decreasing assimilate transport from source leaves to sink organs. Low availability of nitrogen (N) affects plant growth in a highly reminiscent manner to leaf senescence, but can be reverted by the resupply of N. To unravel the critical steps in primary metabolism underlying the growth adjustment in response to low N availability, transcriptomic and comprehensive metabolite analyses were performed in barley using primary leaves at early and later stages of N deprivation, and after N resupply to N-deficient plants.

Development of the work carried out

In order to identify genetic and metabolic factors that regulates leaf senescence in response to whole plant development and N supply, chlorophyll content, root/shoot ratio, hormonal changes, anion levels, C/N ratio, transcriptomic and global metabolic rearrangements were analyzed in barley primary and 4^{th} leaves at different nitrogen regimes. Moreover, phloem exudates of primary leaves were collected in EDTA solution and analyzed for sugar and amino acid levels to monitor export activity. The results showed that leaves undergoing senescence respond to the availability of nitrogen and accordingly to modulate senescence progression and sustain barley plant growth. This is reflected by rearrangements in phytohormone levels mostly cytokinis and abscisic acid, transcriptomic and metabolic responses to leaf development and the leaf N status.
Nitrogen deficiency in leaves caused differential regulation of 1947 genes, mostly belonging to the functional classes photosynthesis, cell wall degradation, lipid degradation, amino acid degradation, transcription factors, phytohormone metabolism and receptor-like kinases. Interestingly, 62% of the genes responding to low N were regulated in the opposite direction after two days of N resupply. Reprogramming of gene transcription was linked to metabolic rearrangements and affected the metabolism of amino acids and sugars. The levels ofmajor amino acids, including Glu, Asp, Ser, Gln, Gly, Thr, Ala and Val, decreased during primary leaf ageand, more pronounced, during low N-induced senescence, which was efficiently reverted after resupply of N. A significant decrease under low N was observed for pyruvate and metabolites involved in the TCA cycle, and this was also reverted after N resupply. Correspondingly, transcript levels of genes coding for pyruvate dehydrogenase, and pyruvate orthophosphate dikinase followed the same trend as related metabolites.

This PhD work showed that in N-deficient barley leaves a specific pathway for remobilization at the link between glycolysis and TCA metabolism is established that is at least partly regulated by a strict reprogramming of the gene coding for pyruvate orthophosphate dikinase. Further analysis of this pathway, its regulatory levels and biochemical changing of pyruvate metabolism enzymes in response to N availability is needed to determine the link between N status and primary metabolism.

Publications

Fataftah N. (2018). A wider perspective on the barley leaf senescence connecting whole plant development and nitrogen availability. (PhD Thesis) Halle/S., Martin-Luther-Universität Halle-Wittenberg, Naturwissenschaftliche Fakultät I Biowissenschaften, 151 pp.

Fataftah N., Mohr C., Hajirezaei M.R., von Wirén N., Humbeck K. (2018). Changes in nitrogen availability lead to a reprogramming of pyruvate metabolism. BMC Plant Biol. 18: 77.

4. Statement as to whether the results of the project are economically exploitable and whether such exploitation will take place or is to be expected / information on possible patents or industrial cooperations

Currently it is not possible to make any statements on the economic exploitation of the results of Part B. Though it is not to be expected that the results will or can be economically exploited without any further research and development.

The results of Part A have no economic exploitation potential in themselves. They serve to perpetuate and continue the IPK Graduate Program.

Various cooperation partners at IPK as well as national and international partners have contributed to some of the studies mentioned above.

5. Press releases and media reports

https://forschung-sachsen-anhalt.de/project/leibniz-graduate-school-gatersleben-17262
https://www.leibniz-gemeinschaft.de/karriere/wissenschaftlicher-nachwuchs/leibniz-graduate-schools/liste/
http://www.sciencecampus-halle.de/index.php/Leibniz_Graduate_Schools.html
https://www.ipk-gatersleben.de/en/aktuelles-2/aktuelle-meldungen/archiv/ipk-aktuell/article/getreideertraege-im-visier/

6. Literature

Maurer A, Draba V, Pillen K (2016) Genomic dissection of plant development and its impact on thousand grain weight in barley through nested association mapping. J Exp Bot 67:2507-2518

Schmalenbach I, Leon J, Pillen K (2009) Identification and verification of QTLs for agronomic traits using wild barley introgression lines. Theor Appl Genet 118:483-497

Schmalenbach I, March TJ, Bringezu T, Waugh R, Pillen K (2011) High-resolution genotyping of wild barley introgression lines and fine-mapping of the threshability locus *thresh-1* using the illumina goldengate assay. G3-Genes Genomes Genetics 1: 187-196

Sharma R, Draicchio F, Bull H, Herzig P, Maurer A, Pillen K, Thomas WTB, Flavell AJ (2018) Genome-wide association of yield traits in a nested association mapping population of barley reveals new gene diversity for future breeding. J Exp Bot 69:3811-3822

7. Workshops

Examples of announced workshops/ lectures from the coursework within the IPK Graduate Program in Annex A