

Transnational Access Report

1. General Information

Project Acronym (ID):	BarleyDwarfs
Project Title	Visual identification of brassinosteroid-deficient mutants in historic barley collections for future crop yield security.
Installation used	IPK-APPP
Name of Group Leader	Dr. Christoph Dockter and Prof. Dr. Mats Hansson
Name of organization	Carlsberg Laboratory, Copenhagen, Denmark

2. Access to the Installation

IPK-APPP

3. Project summary (max. 250 words)

Aim of the project was to perform a comprehensive, high-throughput phenotyping study of nearly 400 uncharacterised, "historic" barley mutants using a state-of-the-art phenotyping module with the ability of visual imaging (LemnaTec facilities, IPK Gatersleben, Germany). Over a period of five weeks the mutant collection was phenotyped automatically and leaf tissue was collected manually for metabolite profiling and for mutant genotyping. The mutant collection was characterized with the application of the LemnaTec platform monitoring several parameters, such as morphological, physiological, colour-related and textural parameters. Apart from the high-throughput, automatic analysis, the mutant collection was also phenotyped manually on the basis of BR-specific morphological features, such as short straw, upright plant architecture with low leaf angles, undulated leaf margins, irregular spike length, shortened awns and an elongated basal rachis internode. All characters were identified earlier in barley BR-deficient mutants induced in different parental backgrounds indicating their robustness. As a result of the project 33 genotypes were selected as potential BR-deficient mutants based on this set of unique phenotypic traits. Parameters detected by the LemnaTec system are currently further evaluated and compared to the results from visual screens. The selected mutants are currently being characterised physiologically in a follow-up experiment, which will allow the mutants' classification. The physiological analysis will be a prerequisite for resequencing of BR-related genes in the selected mutants. Moreover, more than 30 genotypes were selected during the LemnaTec experiment as potential barley *ari-e* and *brh1* mutants, featuring phenotypes very similar to BR-deficient mutants.

4. Main achievements (max. 250 words)

The project implementation allowed automated phenotypic characterization of a large collection (nearly 400 genotypes, four repetitions each) of reduced-height barley mutants and BR-related, genetically characterized mutants (reference genotypes) using a LemnaTec platform. The LemnaTec system enabled visual imaging and gathered data on several plant parameters, such as plant height, width, compactness, colour-related parameters (intensity, hue, colour distribution), as well as fluorescence and NIR-related parameters. The phenotypic analysis was also performed by direct visual screening of the mutants plants based on the set of phenotypic traits which are characteristic for BR-related mutants. Detected data sets are currently being analyzed, interpreted and compared to results of visual screening. At this stage of the project, 33 genotypes were selected as potential BR-deficient mutants based on this set of unique phenotypic traits. Such mutant accessions will be of high scientific value for functional analysis of BR-related genes in cereal crops, including newly identified barley genes such as *HvSTE1* and *HvDWF5*,

(putative involvement in early stages of BR synthesis), which functions have not been validated yet. According to our knowledge this is the first large-scale analysis focusing on selection of BR-related barley mutants derived from various backgrounds. During the plants' growth on the platform leaf tissue samples have been collected from the characterized BR-deficient mutants. The samples are currently being analyzed with the use of the IAP image analysis pipeline (Klukas et al. 2014) to describe metabolite profile of the mutants. This is also a novel approach for physiological and biochemical characterization of barley BR mutants. Apart from the very promising results of the conducted research the project also allowed for establishing a close scientific collaboration between researchers representing several European institutions, such as Carlsberg Laboratory (Denmark), Copenhagen University (Denmark), University of Silesia (Poland) and IPK Gatarsleben (Germany).

Klukas, C., Chen, D., and Pape, J.M. (2014). Integrated Analysis Platform: An Open-Source Information System for High-Throughput Plant Phenotyping. *Plant Physiol* 165, 506-518.

5. Publications related to the access granted, acknowledging the support by EC.

We are currently evaluating the obtained LemnaTec datasets.