

Mining for genes related to climatic stress tolerance in barley by comprehensive quantitative expression analysis

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As the first consequences of the climatic change can be observed by now, the demand for barley varieties tolerant to abiotic stress will soon increase. But as these tolerances are usually of a quantitative nature, it is essential to find the genes involved and to understand the function of these genes, before trying to breed for effective and enduring tolerance.

Experimental setup:



Figure 1: One of the exposition chambers at the Helmholtz Center in Neuherberg, Munich.

The experiment was carried out in the exposition chambers of the Helmholtz Center in Neuherberg, Munich. Three genotypes were tested: Barke, a German spring barley variety with good malting quality, Mut6519, a accession from Uruguay showing a good tolerance to drought stress, and IPZ24727, a breeding line from the Institute of Crop Science and Plant Breeding at the Bavarian State Research Center for Agriculture.

The genotypes were cultivated according to figure 2. Four plant were sown in each tube. The tubes on the right side of each chamber were watered continuously whereas the tubes on the right were submitted to a drought period of 12 days starting at growth stage BBCH 59 (end of heading) for each genotype. In two of the four exposition chambers the light was kept at normal UV level, in the other two the plants were treated with increased UV radiation. Overall the simulated weather inside the chambers mirrored the average of the years 2004-2006, starting at the beginning of April and ending in the middle of August.

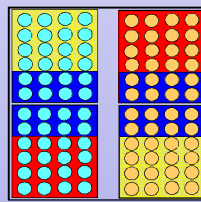


Figure 2: Graphical display of the experimental setup in the exposition chambers: Three genotypes: Barke (red), Mut6519 (blue), IPZ24727 (yellow); Two treatments: constantly watered (light blue) and submitted to drought stress (light brown)

Expression analysis – first round:

Samples were taken at 10 dates throughout the experiment referring to the developmental stage of each genotype and the drought stress period. Always the second leaf from the top of four tillers was cut off and instantly frozen in liquid nitrogen using a different plant for sampling each time.

For the first round of expression analysis aiming at the selection of promising candidate genes for further investigation four sampling dates were chosen to be used in a 44k Agilent Microarray and a 454 sequencing assay.

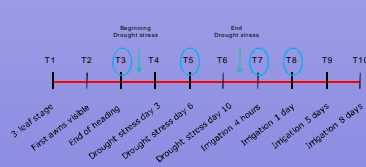


Figure 3: Overview about sampling dates referring to developmental stage and drought stress period. Circled time points have been used for the first round of expression analysis. Beginning and end of the drought stress period are indicated.

44k Agilent Microarray: The Microarray was done at INGM Laboratories GmbH in Munich. For the Microarray sampling dates 3, 5, 7 and 8 were chosen as they cover the whole drought stress period. Samples were submitted from two different tillers per genotype and date only from a chamber with normal UV radiation.

454 sequencing assay: The 454 sequencing assay was carried out by vertis GmbH in Freising-Weißenstephan. For the selection of the samples for the 454 sequencing assay the first results from the Microarray were taken into consideration. Therefore for sampling dates 5 and 7 watered and drought stress exposed samples were included separately. For date 3 samples of both treatments were pooled, for date 8 only the drought stress submitted sample was included. Samples were submitted as a pooled sample from two different tillers per genotype and date from a chamber with normal UV radiation and in addition from a chamber with increased UV radiation.

First results:

Both assays yielded high quality results. Each array hybridisation fulfilled all quality criteria. The sequencing assay resulted in an average of 40.000 reads per sample and 850 bp contig length.

Table 1 gives an overview about the number of deregulated genes in the microarray experiment. Sampling dates 5, 7 and 8 of each genotype and treatment are compared to sampling date 3.

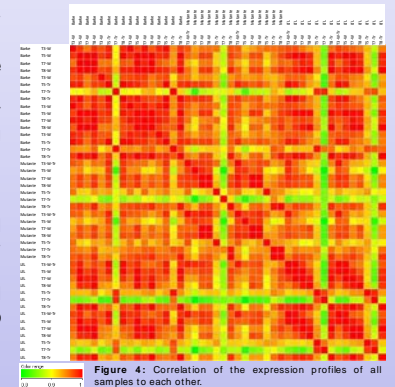


Figure 4: Correlation of the expression profiles of all samples to each other.

Both sets of data are currently analysed at the MPI for Molecular Plant Physiology. After running the usual statistical test with the data, the Gabi PD team will visualize the expression data on barley signalling pathways using the MapMan tool. Thereby coherently regulated genes will be identified and pathways associated with drought stress will be further elucidated.

Another task for the working group is the comparison of both sets of expression data. The first step here will be to bring the Microarray and the 454 data to a common base by aligning the Microarray database with the 454 contigs.

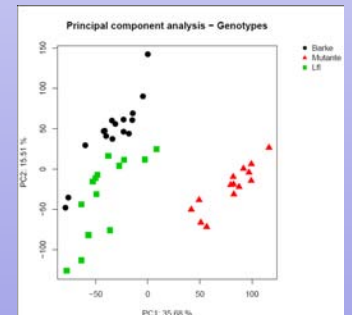


Figure 5: Principal Component Analysis (PCA) of transcript expression values of the 3 genotypes. Normalised signal intensities measured in DNA array experiments (INGM) on samples taken from stressed (drought) and control plants (T3, T5, T7, each) were included in the analysis. A standard principal component (PC) analysis was performed using the R software package for statistical computation (using default parameters: data centered at zero, no unit variance scaling). The plot shows the eigenvalues (as percentages of the total value) of PC 1 plotted against PC 2. The values represent the percentage of the total variance explained by the principal component.

Outlook:

As the aim of this project is to elucidate the pathways involved in drought stress tolerance as well as to identify genes which might be interesting for breeding purposes the most promising candidate genes from comparison within genotypes and between genotypes will be selected. For these genes PCR primers will be designed and they will be validated by real time PCR. In this second round of expression analysis all ten sampling dates for each genotype and treatment will be included.

comparison	PC	Z		down-reg.
		p < 0.001	p < 0.001	
Barke T5 W vs. Barke T3 W	2	62	14	48
Barke T7 W vs. Barke T3 W	2	20	15	14
Barke T5 W vs. Barke T3 W	2	59	16	43
Barke T5 T vs. Barke T3 W	2	30	9	21
Barke T7 T vs. Barke T3 W	2	521	204	207
Barke T5 T vs. Barke T3 W	2	-	-	-
LfL T5 W vs. LfL T3 W	2	36	6	30
LfL T7 W vs. LfL T3 W	2	40	8	41
LfL T5 T vs. LfL T3 W	2	99	24	75
LfL T5 T vs. LfL T3 W	2	132	49	73
LfL T7 T vs. LfL T3 W	2	86	30	47
LfL T5 T vs. LfL T3 W	2	36	13	23
Mutante T5 W vs. Mutante T3 W	2	20	5	24
Mutante T7 W vs. Mutante T3 W	2	64	15	49
Mutante T5 T vs. Mutante T3 W	2	59	16	43
Mutante T5 T vs. Mutante T3 W	2	74	30	38
Mutante T7 T vs. Mutante T3 W	2	61	15	46
Mutante T5 T vs. Mutante T3 W	2	116	41	75

Table 1: Overview of the number of up- and downregulated genes in a selection of comparisons.

Acknowledgement

This project was funded by the Bayerisches Staatsministerium für Ernährung, Landwirtschaft und Forsten (Bay. StMELF), KL/08/06.