

Transnational Access Report

1. General Information

Project Acronym (ID):	BRACHYDROUGHT
Project Title	Brachypodium adaptation to drought stress across different geographic and ecological clines
Installation used	ABER IPC
Name of Group Leader	Pilar Catalan
Name of organization	University of Zaragoza

2. Duration of access

Begin of the project	End of the project
First day the installation was used	Last day the installation was used
9 June 2014	31 December 2014

3. Project summary (max. 250 words)

The main aims of the projects were to assess the variation in water stress responses, through the analysis of several phenomic and metabolomics traits, in *Brachypodium distachyon* lines distributed across different geographic clines in the Iberian Peninsula, and to calibrate the LemnaTec system for small stature plants by comparing the data of two phenotypic traits (plant height, tiller number) obtained from LemnaTec with those retrieved from conventional manual measurements in the *B. distachyon* sampling.

Image derived height measurements from the Lemnatec system introduced high variance, due to growing 4 plants in one tray. Although there were dividers between the plants, the taller and wider plants impinged in the background of neighbouring plants. This could mask ecotype and treatment differences that were observed with individual manual measurements. Manual plant height measurements were able to identify a number of potential height markers in the *B. distachyon* ecotypes sampled. Higher replication could help to reduce the influence of problems with watering regimes over long term drought experiments, allowing outliers to be easily identified.

The Lemnatec system provides a suitable environment in which to grow plants under water controlled conditions for phenotypic analysis and metabolomic experiments. Metabolomic differences between the ecotypes are clearly linked to ecotype location and climate, especially those from warmer southern Spain and Balearic Islands (Menorca) populations when compared to the mesic northern Spain ecotypes. Coumaroylquinic and Feruloylquinic were both negatively correlated with plant height with the taller plants tending to have less of these metabolites.

4. Main achievements (max. 250 words)

Phenomics: Plant height measurements derived from the acquired images showed an irregular trend over time, although the general trends showed that all the ecotypes reached peak height around 30 DUT. Cart positions A and C were most affected by poor watering across all the ecotypes, mainly in the heavily droughted (15% FC) plants. The 75% FC plants were least affected. The image derived plant area measurements also showed similar trends as the height measurements with a strong correlation of 0.775. Manual measurements detected height responses to drought in lines Bd21, Bdis941, B105, Abr6 and Abrc7d. Plants were taller in 75%FC than in 30%FC and 15%FC treatments. The remaining lines did not exhibit this height gradient. No tiller number differences among lines and treatments were detected.

Metabolomics: 3435 and 2044 variables in positive and negative modes, respectively, were obtained. Plants from the 75% FC watering treatment were used to compare the ecotype differences. Samples from southern Spain and Balearic Island populations were strongly differentiated from those from northern Spain populations. LDA also differentiated the reference Bd21 line (Iraq) from the rest of the populations in the 2D plot. This was more prominent in negative mode but was also found in positive mode. Hierarchical Cluster Analysis (HCA) showed a clear differentiation of southern (B111) and island (Bdis941) samples in positive and negative modes. Bd21 was closer to northern Spain than to southern Spain and Menorca samples. This accession showed an intermediate position between the two groups in the positive mode but a closer affinity to northern ecotypes (CAS1, ARN2, SAR2) in the negative mode. When all the ecotypes' samples from the three treatments were combined, a significant difference was found between the 75% FC and 15% FC treatments in LDA. Metabolomic differences between the drought stressed plants (15%FC) and the well watered plants (75%FC) for each line were assessed. LDA found massive differences among lines and treatments (Tw1 10.44/Tw2 5.29). The southern (B111) and Menorca (Bdis941) ecotypes were also differentiated from the rest. The strongest differences were found within the NE Spain Monegros arid ecotype Sar2. Bd21 showed less response to treatments than the expected. Ecotypic RF margins showed metabolomic statistical differences between the 15% and 75% treatments in all the ecotypes studied in positive mode and in 8 out of 9 ecotypes studied in negative mode. No statistical differences were found between 30% and 75% treatments except for Sar2. 190 and 165 potentially explanatory drought features were found in negative and positive mode, respectively. The positive mode data showed similar trends as the negative mode data; however the treatments' effects were more prominent than the ecotypic differences. RF regression analysis of manual plant height means with metabolomic m/z variables yielded an average R² of 0.401 and 0.592 across the 100 re-samples, in positive and negative modes respectively. 37 and 33 explanatory m/z variables were identified as potential plant height markers using variable importance thresholds of 10 and 5 in positive and negative modes, respectively. Putative annotation of these explanatory m/z variables identified a number of lipids and numerous secondary metabolites. The most notable of which were the two phenylpropanoid pathway Coumaroylquinic and Feruloylquinic metabolites.

This work was the first instance of material grown in NPPC undergoing metabolomic analysis in Aberystwyth University's High Resolution Metabolomics Laboratory.

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

Pérez-Collazos E, Finch JP, Corke F, Catalan P, Draper J, Doonan J. 2015. Metabolomic and phenotypic drought stress responses in *Brachypodium distachyon* accessions across a climate gradient. In prep.

Catalan P, López-Alvarez D, Bellosta C, Pérez-Collazos E, Corke F, Mur L, Zubair H, Draper J, Doonan JH. 2013. Phenotypic, genotypic and metabolomic analyses within the *Brachypodium distachyon* complex (Poaceae): first approaches to phenomics and GWA studies. EPPN workshop. Oral presentation. Porto Heli. 05/09/2013.