**Final Project Summary**: TEMPIC (237398)

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The purpose of this project was to assess the impact that changes in ambient temperature, through phenomena such as climate change, are likely to have on monocot crop plants. Specifically, this was done using the model organism *Brachypodium distachyon*, which is a temperate grass closely related to wheat and barley. Three stages of plant development were assessed, including; young vegetative seedlings, vegetative to reproductive transition and the period of endosperm development and starch accumulation during grain filling, with the later being addressed to increase our understanding of how climate change will effect yield production in major crops, which has strong socio-economic impacts.

Firstly, to address the effect that temperature changes have on young seedlings, transcript levels were compared between plants grown at 12ºC to those shifted to either 22ºC or 27ºC, to identify genes that displayed an altered expression pattern proportional to the rise in temperature and to determine the overall response of the transcriptome. Through this analysis, two genes called Heat Shock Protein 70 (HSP70) and Heat Shock Factor 23 (HSF23) were identified as having an increase in gene transcription that was proportional to the rise in temperature. Subsequent analysis showed this result was consistent with plants grown at constant temperatures of 17ºC, 22ºC or 27ºC. This analysis was performed on a genome wide scale using the Brachypodium microarray, with the expression of candidate genes confirmed by quantitative PCR. The microarray revealed 323 genes were unregulated at 27ºC compared to 12ºC, and 344 genes were down-regulated. While this response is robust, the number of genes with altered expression is greatly reduced compared to corresponding experiments in Arabidopsis, suggesting that vegetative tissue of temperate grasses is less responsive than that in dicot plants. This response was further characterised by analysing the behaviour of nucleosomes surrounding the transcription start site (TSS), which had been shown in Arabidopsis to coordinate the transcriptional events that occur as temperature changes. The sensitivity of these particular nucleosomes to changes in temperature is affected by the presence of the histone 2A variant, H2A.Z. Using transgenic lines containing a tagged version of H2A.Z, which were developed during this project, H2A.Z levels and overall nucleosome occupancy at sites surrounding the TSS of these two genes were assessed at 17ºC and 27ºC. These results supported the transcript data, as they suggested that the nucleosomes were not very responsive within this temperature range, which is contrary to results obtained in Arabidopsis. Experiments including tissue from plants grown at 32ºC indicated that a higher ambient temperature did significantly reduce the levels of nucleosomes and H2A.Z.

Included in the analysis of the ambient temperature response in vegetative tissue was an assessment of how temperature effects the transition of Brachypodium from vegetative to reproductive life-cycles. Unlike the well-characterised response of Arabidopsis where temperature has been show to compensate for photoperiod in the initiation of flowering, growth in short-days at 27ºC was not able to promote flowering.

To assess the effect that temperature has on grain development, initial phenotypic analysis was performed on plants that were grown under two different temperature regimes, which were altered as the plants proceeded into the stage of endosperm development and starch accumulation. The temperature regimes were 22°C/17°C (day/night) and 27°C/22°C (D/N). Growing plants at 27°C/22°C resulted in an acceleration through stage of endosperm development, and an early maturity that caused 10-15% reduction in yield relative to 22ºC/17ºC. Also, the higher temperature caused a change in expression of HSF23, as well as genes involved in starch accumulation and defence related processes in the developing grain. The changes in transcript levels were partnered with a reduction in nucleosome occupancy surrounding the TSSs, with these nucleosomes found to contain the H2A variant, H2A.Z, whose presence was also much reduced. These reductions far exceeded those seen in the young vegetative tissue, thus suggesting that developing grain is much more sensitive to changes in ambient temperature compared to vegetative tissue. Taking into account the environments that Brachypodium and other temperate grasses originate from, it is a reasonable conclusion from this analysis that temperate grasses have developed a strategy to tolerate higher ambient temperatures during vegetative stages, but are sensitive to the effects of higher temperatures during grain development. Part of this strategy seems to involve the plants proceeding more rapidly through endosperm development to provide greater opportunity of a viable embryo being produced, prior to the arrival of even hotter temperatures.