



Natural variation in *Brachypodium distachyon* responses to combined abiotic stresses

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The growing world population increases demand for agricultural production, which is becoming even more challenging as climate change increases global temperatures and causes more extreme weather events. Using high-throughput phenotyping, this study examines the phenotypic variation of 149 accessions of *Brachypodium distachyon* under drought, heat, and the combination of both stresses. Heat alone causes the largest amounts of tissue damage and the combination of heat and drought causes the largest decrease in plant biomass compared to other treatments, however, we identified heat alone as being the most detrimental stress condition. Notably, we identified Bd21-0, the reference line for *B. distachyon*, as not having robust growth under stress conditions, especially in the heat-drought combined treatment. We found climate of origin (climate data from the accessions' collection locations) to be significantly associated with height and percent of plant tissue damage under the conditions assessed, indicating a relationship between climate of origin and *B. distachyon* phenotype under drought and heat stresses. Additionally, genome wide association mapping found a number of genetic loci associated with changes in plant height, biomass, and the amount of damaged tissue under stress. Significant SNPs were closely located to genes known to be involved in plant responses to abiotic stresses. The anticipated increase in drought and heat stress as a result of climate change and the distinct impact of stresses in combination, as demonstrated in this study, underscores the importance of phenotyping plants under multiple stresses that frequently converge.