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48	2) F	xnerimental design details
49	The 1	parents and F, hybrids were planted in the field on October 12, 2012 as part of a larger
50	expe	riment. The field experimental site was located in a prairie field (30.182° N, 97.879° W) at

51 the south end of the Ladybird Johnson Wildflower Center (Austin, TX). Prior to planting, the

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52 field was covered with Sunbelt Weed Fabric (Peaceful Valley Farms, Grass Valley, CA). For

planting, we cut holes in the cloth and used a mechanical auger to drill holes in the soil. Plants
were transferred from 3.79L pots into the holes. Plants were arrayed into rows that were two

55 plants wide, with 1.2m wide walking paths between rows to allow for easy access to plants for

56 sampling. Plants were spaced 40 cm apart from each other along rows. The full design included

57 1000 fully randomized plants, of which the parents (N = 100 FIL2, 100 HAL2) and F_{1s} (N = 30)

58 were a subset. Due to exceptionally low rainfall in the fall of 2012, we watered plants as needed 59 through November and early December to ensure establishment. Irrigation was ceased once

60 plants entered winter dormancy. The experimental plants emerged from dormancy in the spring of

61 2013 and were grown without manipulation until early July. Plants grew vigorously through April

and May, as rainfall was abundant in those months (LBJ Wildflower Center Weather Station

Data: April = 80.0 mm; May = 93.5 mm; Fig. 1). However, this period was followed by a
 prolonged hot and dry period, where only 5.08 mm of rainfall fell between 26-May and 7-July.

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67 3) Assessment of experimental conditions:

Daily maximum air temperature and total precipitation were requested and downloaded from the
 National Oceanic and Atmospheric Administration (NOAA) web portal

(https://www.ncdc.noaa.gov/cdo-web/) the most proximate weather stations to the collections
sites of HAL2 and FIL2. Data for each year over the span of the experiment were parsed from
these data files. Kernel density distributions for each climate variable were calculated using the R
base function *density* and the percentile distribution of the 2013 drought were compared for each
dataset. Local weather station data was available for 2013 at the site of the experiment. These

75 data augmented our broader scale climate data.

76 77

78 4) Processing of counts data:

79 Libraries were compared by the relative expression of HAL2- and FIL2- allele-specific-80 expression (ASE). Lines with studentized residuals from the linear model (HAL2 ASE ~ FIL2 81 ASE) within each genotype (HAL2, FIL2, and F_1) that gave bonferoni-adjusted p-values (t test) < 82 0.0001 were excluded from all further analyses. This was accomplished using the function 83 outlierTest in the R package car (Fox and Weisberg 2010). Genes with mean counts across all 84 samples < 5 were also excluded from all further analyses. We then assessed ASE of the genes 85 with >5 total counts and culled by two criteria. For a gene to be incorporated into our modeling of 86 cis- and trans- acting expression regulation, genes had to have >5 mean FIL2 ASE counts in the 87 FIL2 genotype and >5 mean HAL2 ASE counts in the HAL2 genotype. Furthermore, the mean 88 HAL2 ASE counts in FIL2 genotypes and the mean FIL2 ASE counts in HAL2 genotypes had to 89 be >1% of the FIL2 ASE counts in the FIL2 genotype and the HAL2 ASE counts in the HAL2 90 genotype respectively. These criteria assured that all genes analyzed had sufficiently precise and 91 quantifiable ASE.

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94 5) Estimation of mapping bias: Many analysis of ASE are sensitive to mapping bias. We 95 corrected for these effects by treating ASE from each plant and allele as its own library in the 96 DESeq2 pipeline. As such, the FIL2 and HAL2 ASE are each normalized with regards to the total 97 number of counts assigned to each bin. Despite these efforts, extreme mapping bias may still 98 introduce statistical artifacts into our analysis. To test mapping bias, we compared the mean 99 number of mapped reads for each allele, for each gene. If the linear correlation falls near a slope 100 of 1, this is an indication that mapping bias is not extreme. Indeed, this is the case (Fig. S6). We 101 find very little evidence of mapping bias.

102

60 Model Specification:Our model fit treatment (drough, recovery), genotype (FIL2, HAL2) and their interaction whileControlling for sampling scheme. Assessment of differential expression was conducted by fittingtwo distinct models in <i>DESeq2</i> , one with total counts as the response variable and another withallcle-specific counts (ASE). In each model, we corrected for the time of sampling bycategorizing tissue harvest as early (in the first 50% of sampling) or late (in the last 50% ofsampling). This categorical time variable was retained as the first term in all DESeq2 models toaccount for circadian variation in the linear models.In the first model, we quantified differential expression in the context of genotype, environmentand GxE between the parental genotypes, FIL2 and HAL2 (Fig. 2a). Genes were retained for theanal GxE between the parental genotypes, FIL2 and HAL2 (Fig. 2a). Genes were retained for theanal GxE between the parental genotypes, FIL2 and HAL2 (Fig. 2a). Genes were retained for theanal GxE between the parental genotypes, FIL2 and HAL2 (Fig. 2a). Genes were retained for theanal GxE between the gravity binomial linear model DESeq2 pipeline (Love et al. 2013; 2014)with:model in the day, <i>T_i</i> = 1 if the sample was collected late in the day and 0 it wascollected early in the day, <i>T_i</i> = 1 if the sample was collected late in the day and 0 if it is adrought treatment sample, and <i>A_i</i> = 1 if the sample genotype and hild fifters between theenvironments and/or genotypes, or genes that are differentially affected by the environment,depending on the genotype (GxE).We quantified heterosis by including total counts of F, genotypes in	103	
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108 allele-specific counts (ASE). In each model, we corrected for the time of sampling by categorizing tissue harvest as early (in the first 50% of sampling) or late (in the last 50% of account for circadian variation in the linear models.111 112 113In the first model, we quantified differential expression in the context of genotype, environment and GxE between the parental genotypes, FIL2 and HAL2 (Fig. 2a). Genes were retained for the analysis if mean total counts were > 5, resulting in a total of 22,322 genes analyzed. For each gene we fit the standard negative binomial linear model DESeq2 pipeline (Love et al. 2013; 2014) with:112 114 115MODEL 1: log; $q_{ij} = \beta_0 + \beta_0 D_i + \beta_0 T_i + \beta_0 A_i + \beta_0 T_i^* A_i$ 112 114 115MODEL 1: log; $q_{ij} = \beta_0 + \beta_0 D_i + \beta_0 T_i + \beta_0 A_i + \beta_0 T_i^* A_i$ 112 116 117 118 118 118MODEL 1: log; $q_{ij} = \beta_0 + \beta_0 D_i + \beta_0 T_i + \beta_0 A_i + \beta_0 T_i^* A_i$ 112 118 119 119 110 1117 1118 1110 1111 1110 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 111111 11111 11111 11111 11111 11111 	107	two distinct models in <i>DESeq2</i> , one with total counts as the response variable and another with
109categorizing tissue harvest as early (in the first 50% of sampling) or late (in the last 50% of sampling). This categorical time variable was retained as the first term in all DESeq2 models to account for circadian variation in the linear models.111 112 113 114In the first model, we quantified differential expression in the context of genotype, environment and GxE between the parental genotypes, FIL2 and HAL2 (Fig. 2a). Genes were retained for the analysis if mean total counts were > 5, resulting in a total of 22,322 genes analyzed. For each gene we fit the standard negative binomial linear model DESeq2 pipeline (Love et al. 2013; 2014) with:118 119 110 111 11111 111111 111111 11111 111111 111111 111111 111111 111111 1111111 111111 111111 111111 111111 1111111	108	allele-specific counts (ASE). In each model, we corrected for the time of sampling by
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analysis if mean total counts were > 5, resulting in a total of 22,322 genes analyzed. For each gene we fit the standard negative binomial linear model DESeq2 pipeline (Love et al. 2013; 2014) with: MODEL 1: $log_2 q_0 = \beta_0 + \beta_0 D_i + \beta_T T_i + \beta_4 A_i + \beta_{Ta} T_i^* A_i$ where for individual i and gene j, $D_i = 1$ if the sample was collected late in the day and 0 it was collected early in the day, $T_i = 1$ if the sample genotype allele is HAL2 and 0 if it is FIL2. This analysis permitted the estimation of conserved genes that have similar responses across all individuals and environments (conserved), genes with expression that differs between the environments and/or genotypes, or genes that are differentially affected by the environment, depending on the genotype (GxE). We quantified heterosis by including total counts of F ₁ genotypes in MODEL 1. As such this model is a simple expansion of Model 1, where A has three levels (HAL2, FIL2 and F ₁). From this model, we conducted specific contrasts between the there genotypes and binned each gene into one of the seven heterosis categories (Table 1) via a custom R script. To quantify the degree of cis- and trans-acting expression modifiers, we again fit the standard DESeq2 model to allele-specific expression of HAL2, FIL2 and the F ₁ hybrid, but with: MODEL 2: $log_2 q_u = \beta_0 + \beta_0 D_i + \beta_T T_i + \beta_4 A_i + \beta_6 G_i + \beta_{Ta} T_i^* A_i + \beta_{Ta} T_i^* G_i + \beta_{Ac} A_i^* G_i + \beta_{Ta} G_i^* A_i^* G_i$ where for individual <i>i</i> and gene <i>j</i> , $D_i = 1$ if the sample was collected late in the day and 0 it was collected early in the day, $T_i = 1$ if the sample was collected late in the day and 0 it was collected early in the day, $T_i = 1$ if the count is for the HAL2 allele and $G_i = 1$ if the sample from the F_0 generation and 0 if it is from the drought treatment, $A_i = 1$ if the count is for the HAL2 allele and 0 if the count is for the FIL2 allele and $G_i = 1$ if the sample from the F_0 generation and 0 if it is from the F_1 generatio	114	and GxE between the parental genotypes. FIL2 and HAL2 (Fig. 2a). Genes were retained for the
116 gene we fit the standard negative binomial linear model DESeq2 pipeline (Love et al. 2013; 2014) 117 with: 118 119 MODEL 1: 120 $log_2 q_{ij} = \beta_{\theta} + \beta_{\rho} D_i + \beta_{\tau} T_i + \beta_{A} A_i + \beta_{Ta} T_i^* A_i$ 121 where for individual i and gene j, $D_i = 1$ if the sample was collected late in the day and 0 it was 123 collected early in the day, $T_i = 1$ if the sample genotype allele is HAL2 and 0 if it is FIL2. 124 drought treatment sample, and $A_i = 1$ if the sample genotype allele is HAL2 and 0 if it is FIL2. 125 This analysis permitted the estimation of conserved genes that have similar responses across all 126 individuals and environments (conserved), genes with expression that differs between the 127 environments and/or genotypes, or genes that are differentially affected by the environment, 128 depending on the genotype (GxE). 130 We quantified heterosis by including total counts of F_1 genotypes in MODEL 1. As such this 131 model is a simple expansion of Model 1, where A has three levels (HAL2, FIL2 and F ₁). From 132 this model, we conducted specific contrasts between the three genotypes and binned each gene 134 into one of the seven heterosis categories (Table 1) via a custom R script. 135 136 To quantify the degree of cis- and trans-acting expression modifiers, we again fit the standard 137 DESeq2 model to allele-specific expression of HAL2, FIL2 and the F ₁ hybrid, but with: 138 140 $MODEL 2:$ 140 $log_2 q_{ij} = \beta_0 + \beta_0 D_i + \beta_7 T_i + \beta_A A_i + \beta_C G_i + \beta_{TA} T_i^* A_i + \beta_{TC} T_i^* G_i + \beta_{AC} A_i^* G_i + \beta_{TAC} T_i^* A_i^* G_i$ 141 where for individual <i>i</i> and gene <i>j</i> , $D_i = 1$ if the sample was collected late in the day and 0 it was 142 collected early in the day, $T_i = 1$ if the count is for the HAL2 allele and 0 if it is from 144 the drought treatment, $A_i = 1$ if the count is for the HAL2 allele and 0 if the count is for the FIL2 145 allele and $G_i = 1$ if the sample from the F_0 generation and 0 if it is from the fugeneration. 146 147 Under th	115	analysis if mean total counts were > 5 resulting in a total of 22 322 genes analyzed. For each
with: With: MODEL 1: $log_2 q_{ij} = \beta_0 + \beta_D D_i + \beta_T T_i + \beta_A A_i + \beta_{TA} T_i^* A_i$ where for individual i and gene j, $D_i = 1$ if the sample was collected late in the day and 0 it was collected early in the day, $T_i = 1$ if the sample is a recovery treatment sample and 0 if it is a drought treatment sample, and $A_i = 1$ if the sample genotype allele is HAL2 and 0 if it is FIL2. This analysis permitted the estimation of conserved genes that have similar responses across all individuals and environments (conserved), genes with expression that differs between the environments and/or genotypes, or genes that are differentially affected by the environment, depending on the genotype (GXE). We quantified heterosis by including total counts of F ₁ genotypes in MODEL 1. As such this model is a simple expansion of Model 1, where A has three levels (HAL2, FIL2 and F ₁). From this model, we conducted specific contrasts between the three genotypes and binned each gene into one of the seven heterosis categories (Table 1) via a custom R script. To quantify the degree of cis- and trans-acting expression modifiers, we again fit the standard DESeq2 model to allele-specific expression of HAL2, FIL2 and the F ₁ hybrid, but with: MODEL 2: $log_2 q_{ij} = \beta_0 + \beta_0 D_i + \beta_T T_i + \beta_A A_i + \beta_C G_i + \beta_{TA} T_i^* A_i + \beta_{TC} T_i^* G_i + \beta_{AC} A_i^* G_i + \beta_{TAC} T_i^* A_i^* G_i$ where for individual <i>i</i> and gene <i>j</i> , $D_i = 1$ if the sample was collected late in the day and 0 it was collected early in the day, $T_i = 1$ if the count is for the HAL2 allele and 0 if it is from the drought treatment, $A_i = 1$ if the count is for the HAL2 allele and 0 if it is from the drought treatment, $A_i = 1$ if the count is for the HAL2 allele and 0 if it is from the drought treatment, $A_i = 1$ if the count is for the HAL2 allele and 0 if it is from the drought treatment, $A_i = 1$ if the count is for the HAL2 allele and 0 if the semation. Haft the MODEL 2 specification, β_A estimates the allele contra	116	gene we fit the standard negative binomial linear model DESeq2 pineline (Love et al. 2013: 2014)
MODEL 1: 10 $log_2 q_{ij} = \beta_0 + \beta_0 D_i + \beta_T T_i + \beta_A A_i + \beta_T A_i T_i^* A_i$ 12 where for individual i and gene j, $D_i = 1$ if the sample was collected late in the day and 0 it was 12 collected early in the day, $T_i = 1$ if the sample is a recovery treatment sample and 0 if it is a 12 drought treatment sample, and $A_i = 1$ if the sample genotype allele is HAL2 and 0 if it is FIL2. 12 This analysis permitted the estimation of conserved genes that have similar responses across all 12 individuals and environments (conserved), genes with expression that differs between the 12 environments and/or genotypes, or genes that are differentially affected by the environment, 12 depending on the genotype (GxE). 13 We quantified heterosis by including total counts of F ₁ genotypes in MODEL 1. As such this 14 model is a simple expansion of Model 1, where A has three levels (HAL2, FIL2 and F ₁). From 15 this model, we conducted specific contrasts between the three genotypes and binned each gene 16 into one of the seven heterosis categories (Table 1) via a custom R script. 17 o quantify the degree of cis- and trans-acting expression modifiers, we again fit the standard 17 DESeq2 model to allele-specific expression of HAL2, FIL2 and the F ₁ hybrid, but with: 18 MODEL 2: 10 $log_2 q_{ij} = \beta_0 + \beta_0 D_i + \beta_T T_i + \beta_A A_i + \beta_G G_i + \beta_T A_i^* A_i + \beta_T G_i^* F_A + \beta_A G_i^* F_A + \beta_T G_i$	117	with.
119MODEL 1:120 $log_2 q_{ij} = \beta_0 + \beta_D D_i + \beta_T T_i + \beta_A A_i + \beta_T A_i^* A_i$ 121where for individual i and gene j, $D_i = 1$ if the sample was collected late in the day and 0 it was123collected early in the day, $T_i = 1$ if the sample is a recovery treatment sample and 0 if it is a124drought treatment sample, and $A_i = 1$ if the sample genotype allele is HAL2 and 0 if it is FIL2.125This analysis permitted the estimation of conserved genes that have similar responses across all126individuals and environments (conserved), genes with expression that differs between the127environments and/or genotypes, or genes that are differentially affected by the environment,128depending on the genotype (GxE).130We quantified heterosis by including total counts of F ₁ genotypes in MODEL 1. As such this132model is a simple expansion of Model 1, where A has three levels (HAL2, FIL2 and F ₁). From133this model, we conducted specific contrasts between the three genotypes and binned each gene134into one of the seven heterosis categories (Table 1) via a custom R script.135To quantify the degree of cis- and trans-acting expression modifiers, we again fit the standard137DESeq2 model to allele-specific expression of HAL2, FIL2 and the F ₁ hybrid, but with:138MODEL 2:140 $log_2 q_{ij} = \beta_0 + \beta_0 D_i + \beta_T T_i + \beta_A A_i + \beta_C G_i + \beta_T A_i^* A_i + \beta_T G_i + \beta_A CA_i^* G_i + \beta_{Ta} G_i^* A_i^* G_i$ 141where for individual i and gene j, $D_i = 1$ if the sample was collected late in the day and 0 it was141collected early in the day,	118	
$log_2 q_{ij} = \beta_0 + \beta_0 D_i + \beta_T T_i + \beta_A A_i + \beta_{TA} T_i^* A_i$ $log_2 q_{ij} = \beta_0 + \beta_0 D_i + \beta_T T_i + \beta_A A_i + \beta_{TA} T_i^* A_i$ where for individual i and gene j, $D_i = 1$ if the sample was collected late in the day and 0 if was collected early in the day, $T_i = 1$ if the sample genotype allele is HAL2 and 0 if it is FIL2. This analysis permitted the estimation of conserved genes that have similar responses across all individuals and environments (conserved), genes with expression that differs between the environments and/or genotypes, or genes that are differentially affected by the environment, depending on the genotype (GxE). We quantified heterosis by including total counts of F ₁ genotypes in MODEL 1. As such this model is a simple expansion of Model 1, where A has three levels (HAL2, FIL2 and F ₁). From this model, we conducted specific contrasts between the three genotypes and binned each gene into one of the seven heterosis categories (Table 1) via a custom R script. To quantify the degree of cis- and trans-acting expression modifiers, we again fit the standard DESeq2 model to allele-specific expression of HAL2, FIL2 and the F ₁ hybrid, but with: log ₂ $q_{ij} = \beta_0 + \beta_0 D_i + \beta_T T_i + \beta_A A_i + \beta_C G_i + \beta_{TA} T_i^* A_i + \beta_{TC} T_i^* G_i + \beta_{Ac} A_i^* G_i + \beta_{TA} T_i^* A_i^* G_i$ where for individual <i>i</i> and gene <i>j</i> , $D_i = 1$ if the sample was collected late in the day and 0 it was collected early in the day, $T_i = 1$ if the sample is from the recovery treatment and 0 if it is from the drought treatment, $A_i = 1$ if the count is for the HAL2 allele and 0 if the count is for the FIL2 allele and $G_i = 1$ if the sample from the F_0 generation and 0 if it is from the F ₁ generation. Under the MODEL 2 specification, β_A estimates the allele contrast within the F ₁ generation, which corresponds to the classic cis-test notion of allelic imbalance: A(F ₁)/B(F ₁). The interaction term β_{AG} can be interpreted as a difference of differences the difference between alle	119	MODEL 1
121 122 where for individual i and gene j, $D_i = 1$ if the sample was collected late in the day and 0 it was 123 collected early in the day, $T_i = 1$ if the sample genotype allele is HAL2 and 0 if it is FIL2. 124 drought treatment sample, and $A_i = 1$ if the sample genotype allele is HAL2 and 0 if it is FIL2. 125 126 This analysis permitted the estimation of conserved genes that have similar responses across all 127 individuals and environments (conserved), genes with expression that differs between the 128 environments and/or genotypes, or genes that are differentially affected by the environment, 129 depending on the genotype (GxE). 130 131 We quantified heterosis by including total counts of F ₁ genotypes in MODEL 1. As such this 132 model is a simple expansion of Model 1, where A has three levels (HAL2, FIL2 and F ₁). From 133 this model, we conducted specific contrasts between the three genotypes and binned each gene 134 into one of the seven heterosis categories (Table 1) via a custom R script. 135 136 137 138 139 139 140 141 141 141 141 141 141 141	120	$log_{\alpha} = R_{\alpha} + R_{\alpha} D + R_{\alpha} T + R_{\alpha} A + R_{\alpha} T + A$
where for individual i and gene j, $D_i = 1$ if the sample was collected late in the day and 0 it was collected early in the day, $T_i = 1$ if the sample is a recovery treatment sample and 0 if it is a drought treatment sample, and $A_i = 1$ if the sample genotype allele is HAL2 and 0 if it is FIL2. This analysis permitted the estimation of conserved genes that have similar responses across all individuals and environments (conserved), genes with expression that differs between the environments and/or genotypes, or genes that are differentially affected by the environment, depending on the genotype (GxE). We quantified heterosis by including total counts of F ₁ genotypes in MODEL 1. As such this model is a simple expansion of Model 1, where A has three levels (HAL2, FIL2 and F ₁). From this model, we conducted specific contrasts between the three genotypes and binned each gene into one of the seven heterosis categories (Table 1) via a custom R script. To quantify the degree of cis- and trans-acting expression modifiers, we again fit the standard DESeq2 model to allele-specific expression of HAL2, FIL2 and the F ₁ hybrid, but with: $\log_2 q_{ij} = \beta_0 + \beta_0 D_i + \beta_7 T_i + \beta_A A_i + \beta_C G_i + \beta_{7A} T_i^* A_i + \beta_{7C} T_i^* G_i + \beta_A A_i^* G_i + \beta_{7AG} T_i^* A_i^* G_i$ where for individual <i>i</i> and gene <i>j</i> , $D_i = 1$ if the sample was collected late in the day and 0 it was collected early in the day, $T_i = 1$ if the sample is from the recovery treatment and 0 if it is from the drought treatment, $A_i = 1$ if the count is for the HAL2 allele and 0 if the count is for the FIL2 allele and $G_i = 1$ if the sample from the F ₀ generation and 0 if it is from the f ₁ generation. Under the MODEL 2 specification, β_A estimates the allele contrast within the F ₁ generation, which corresponds to the classic cis-test notion of allelic imbalance: $A(F_1)/B(F_1)$. The interaction term β_{AG} can be interpreted as a difference of differences - the difference between allele contrasts	120	$\log_2 q_{ij} = p_0 + p_D \boldsymbol{\nu}_i + p_T \boldsymbol{\Gamma}_i + p_A \boldsymbol{\Lambda}_i + p_T \boldsymbol{\Lambda}_i \boldsymbol{\Lambda}_i$
where for individual r and gene <i>j</i> , $p_i = 1$ if the sample was collected rate in the day and 0 if it is a collected early in the day, $T_i = 1$ if the sample is a recovery treatment sample and 0 if it is FIL2. This analysis permitted the estimation of conserved genes that have similar responses across all individuals and environments (conserved), genes with expression that differs between the environments and/or genotypes, or genes that are differentially affected by the environment, depending on the genotype (GxE). We quantified heterosis by including total counts of F ₁ genotypes in MODEL 1. As such this model is a simple expansion of Model 1, where A has three levels (HAL2, FIL2 and F ₁). From this model, we conducted specific contrasts between the three genotypes and binned each gene into one of the seven heterosis categories (Table 1) via a custom R script. To quantify the degree of cis- and trans-acting expression modifiers, we again fit the standard DESeq2 model to allele-specific expression of HAL2, FIL2 and the F ₁ hybrid, but with: MODEL 2: $log_2 q_{ij} = \beta_0 + \beta_D D_i + \beta_T T_i + \beta_A A_i + \beta_C G_i + \beta_{TA} T_i^* A_i + \beta_{TC} T_i^* G_i + \beta_A C A_i^* G_i + \beta_{TAC} T_i^* A_i^* G_i$ where for individual <i>i</i> and gene <i>j</i> , $D_i = 1$ if the sample was collected late in the day and 0 it was collected early in the day, $T_i = 1$ if the sample is from the recovery treatment and 0 if it is from the drought treatment, $A_i = 1$ if the count is for the HAL2 allele and 0 if the count is for the FIL2 allele and $G_i = 1$ if the sample from the F ₀ generation and 0 if it is from the F ₁ generation. 447 447 447 447 447 447 447 447 447 44	121	where for individual i and gone i, $D = 1$ if the complexies collected lete in the day and 0 it was
123 collected early in the day, $T_i = 1$ if the sample is a recovery treatment sample and 0 if it is a 124 drought treatment sample, and $A_i = 1$ if the sample genotype allele is HAL2 and 0 if it is FIL2. 125 126 This analysis permitted the estimation of conserved genes that have similar responses across all 127 individuals and environments (conserved), genes with expression that differs between the 128 environments and/or genotypes, or genes that are differentially affected by the environment, 129 depending on the genotype (GxE). 130 131 We quantified heterosis by including total counts of F ₁ genotypes in MODEL 1. As such this 132 model is a simple expansion of Model 1, where A has three levels (HAL2, FIL2 and F ₁). From 133 this model, we conducted specific contrasts between the three genotypes and binned each gene 134 into one of the seven heterosis categories (Table 1) via a custom R script. 135 136 To quantify the degree of cis- and trans-acting expression modifiers, we again fit the standard 137 DESeq2 model to allele-specific expression of HAL2, FIL2 and the F ₁ hybrid, but with: 138 139 MODEL 2: 140 $log_2 q_{ij} = \beta_0 + \beta_0 D_i + \beta_T T_i + \beta_A A_i + \beta_C G_i + \beta_{TA} T_i^* A_i + \beta_{TC} T_i^* G_i + \beta_{AC} A_i^* G_i + \beta_{TAC} T_i^* A_i^* G_i$ 141 where for individual <i>i</i> and gene <i>j</i> , $D_i = 1$ if the sample was collected late in the day and 0 it was 142 collected early in the day, $T_i = 1$ if the sample is from the recovery treatment and 0 if it is from 144 the drought treatment, $A_i = 1$ if the count is for the HAL2 allele and 0 if the count is for the FIL2 146 147 under the MODEL 2 specification, β_A estimates the allele contrast within the F ₁ generation. 148 149 Under the MODEL 2 specification, β_A estimates the allele contrast within the F ₁ generation. 149 β_{AC} can be interpreted as a difference of differences the difference between allele contrasts	122	where for individual 1 and gene J, $D_i = 1$ if the sample was confected fate in the day and 0 it was
drought treatment sample, and $A_i = 1$ if the sample genotype allele is HAL2 and 0 if it is FIL2. This analysis permitted the estimation of conserved genes that have similar responses across all individuals and environments (conserved), genes with expression that differs between the environments and/or genotypes, or genes that are differentially affected by the environment, depending on the genotype (GxE). We quantified heterosis by including total counts of F ₁ genotypes in MODEL 1. As such this model is a simple expansion of Model 1, where A has three levels (HAL2, FIL2 and F ₁). From this model, we conducted specific contrasts between the three genotypes and binned each gene into one of the seven heterosis categories (Table 1) via a custom R script. To quantify the degree of cis- and trans-acting expression modifiers, we again fit the standard DESeq2 model to allele-specific expression of HAL2, FIL2 and the F ₁ hybrid, but with: MODEL 2: $log_2 q_{ij} = \beta_0 + \beta_0 D_i + \beta_T T_i + \beta_A A_i + \beta_C G_i + \beta_{TA} T_i^* A_i + \beta_{TC} T_i^* G_i + \beta_{AC} A_i^* G_i + \beta_{TA} T_i^* A_i^* G_i$ where for individual <i>i</i> and gene <i>j</i> , $D_i = 1$ if the sample was collected late in the day and 0 it was collected early in the day, $T_i = 1$ if the count is for the HAL2 allele and 0 if the count is for the FIL2 allele and $G_i = 1$ if the sample from the F_0 generation and 0 if it is from the F ₁ generation. Under the MODEL 2 specification, β_A estimates the allele contrast within the F ₁ generation, which corresponds to the classic cis-test notion of allelic imbalance: A(F ₁)/B(F ₁). The interaction term β_{AC} can be interpreted as a difference of differences the difference between allele contrasts	123	collected early in the day, $I_i = 1$ if the sample is a recovery treatment sample and 0 if it is a
This analysis permitted the estimation of conserved genes that have similar responses across all individuals and environments (conserved), genes with expression that differs between the environments and/or genotypes, or genes that are differentially affected by the environment, depending on the genotype (GxE). We quantified heterosis by including total counts of F ₁ genotypes in MODEL 1. As such this model is a simple expansion of Model 1, where A has three levels (HAL2, FIL2 and F ₁). From this model, we conducted specific contrasts between the three genotypes and binned each gene into one of the seven heterosis categories (Table 1) via a custom R script. To quantify the degree of cis- and trans-acting expression modifiers, we again fit the standard DESeq2 model to allele-specific expression of HAL2, FIL2 and the F ₁ hybrid, but with: $\log_2 q_{ij} = \beta_0 + \beta_D D_i + \beta_T T_i + \beta_A A_i + \beta_C G_i + \beta_{TA} T_i^* A_i + \beta_{TC} T_i^* G_i + \beta_{AC} A_i^* G_i + \beta_{TAG} T_i^* A_i^* G_i$ where for individual <i>i</i> and gene <i>j</i> , $D_i = 1$ if the sample was collected late in the day and 0 it was collected early in the day, $T_i = 1$ if the sample is from the recovery treatment and 0 if it is from the drought treatment, $A_i = 1$ if the count is for the HAL2 allele and 0 if the count is for the FIL2 allele and $G_i = 1$ if the sample from the F ₀ generation and 0 if it is from the F ₁ generation. Under the MODEL 2 specification, β_A estimates the allele contrast within the F ₁ generation, which corresponds to the classic cis-test notion of allelic imbalance: A(F ₁)/B(F ₁). The interaction term β_{AG} can be interpreted as a difference of differences the difference between allele contrasts	124	drought treatment sample, and $A_i = 1$ if the sample genotype allele is HAL2 and 0 if it is FIL2.
This analysis permitted the estimation of conserved genes that have similar responses across all individuals and environments (conserved), genes with expression that differs between the environments and/or genotypes, or genes that are differentially affected by the environment, depending on the genotype (GxE). We quantified heterosis by including total counts of F ₁ genotypes in MODEL 1. As such this model is a simple expansion of Model 1, where A has three levels (HAL2, FIL2 and F ₁). From this model, we conducted specific contrasts between the three genotypes and binned each gene into one of the seven heterosis categories (Table 1) via a custom R script. To quantify the degree of cis- and trans-acting expression modifiers, we again fit the standard DESeq2 model to allele-specific expression of HAL2, FIL2 and the F ₁ hybrid, but with: $\log_2 q_{ii} = \beta_0 + \beta_D D_i + \beta_T T_i + \beta_A A_i + \beta_G G_i + \beta_{TA} T_i^* A_i + \beta_{TG} T_i^* G_i + \beta_{AG} A_i^* G_i + \beta_{TAG} T_i^* A_i^* G_i$ where for individual <i>i</i> and gene <i>j</i> , $D_i = 1$ if the sample was collected late in the day and 0 it was collected early in the day, $T_i = 1$ if the sample is from the recovery treatment and 0 if it is from the drought treatment, $A_i = 1$ if the count is for the HAL2 allele and 0 if the count is for the FIL2 allele and $G_i = 1$ if the sample from the F ₀ generation and 0 if it is from the F ₁ generation. Under the MODEL 2 specification, β_A estimates the allele contrast within the F ₁ generation, which corresponds to the classic cis-test notion of allelic imbalance: $A(F_1)/B(F_1)$. The interaction term β_{AG} can be interpreted as a difference of differences the difference between allele contrasts	125	
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allele and $G_i = 1$ if the sample from the F_0 generation and 0 if it is from the F_1 generation. 146 147 148 147 148 148 149 148 149 β_{AG} can be interpreted as a difference of differences the difference between allele contrasts 150 145 145 145 145 146 147 148 149 149 149 149 140 149 140 149 140 140 140 140 140 140 140 140	144	the drought treatment, $A_i = 1$ if the count is for the HAL2 allele and 0 if the count is for the FIL2
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147 Under the MODEL 2 specification, β_A estimates the allele contrast within the F ₁ generation, which 148 corresponds to the classic cis-test notion of allelic imbalance: A(F ₁)/B(F ₁). The interaction term 149 β_{AG} can be interpreted as a difference of differences the difference between allele contrasts 150 between generations which corresponds to the classic trans test notion:	146	
148 corresponds to the classic cis-test notion of allelic imbalance: $A(F_1)/B(F_1)$. The interaction term 149 β_{AG} can be interpreted as a difference of differences the difference between allele contrasts 150 between generations which corresponds to the classic trans test notion:	147	Under the MODEL 2 specification, β_4 estimates the allele contrast within the F ₁ generation which
149 β_{AG} can be interpreted as a difference of differences the difference between allele contrasts 150 between generations which corresponds to the classic trans text notion:	148	corresponds to the classic cis-test notion of allelic imbalance: $A(F_1)/B(F_1)$ The interaction term
150 between generations which corresponds to the closed trans text notion:	149	β_{AG} can be interpreted as a difference of differences the difference between allele contrasts
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151 $[A(F_1)/B(F_1)]/[A(F_0)/B(F_0)]$. We also fit MODEL 2 within each environment, to present the 152 similarity of our analysis to traditional cis-trans tests (Figs. S4-5). 153 154 It is important to note that the estimation of cis/trans expression regulation is not identical to 155 measuring differential expression. The test of allelic imbalance (Fig. 2a) is a specific contrast: 156 157 allele = FIL2 & generation = F_0 (FIL2- F_0) vs. HAL2- F_0 . 158 159 However, the cis-test is coded so that we contrast: 160 161 FIL2-F_{1-ASE} vs. HAL2-F_{1-ASE}, 162 163 and the trans test assesses the allele*generation interaction by contrasting the dependency of 164 allelic imbalance on generation so that: 165 166 slope(FIL2-F_{1-ASE} vs. HAL2-F_{1-ASE}) vs. slope(FIL2-F_{0-ASE} vs. HAL2-F_{0-ASE}). 167 168 Finally, it is important to define the cases where this approach lacks power to detect cis, or trans 169 effects. The most common pattern occurs where significant differential expression exists between 170 the parental genotypes and differential expression exists in the same direction, but is no longer 171 significant in the F_1 generation. In this case, the cis and trans effects are antagonistic, causing our 172 model to detect differential expression between the parents. However, we would be unable to 173 attribute this divergence to either cis, or trans effects. In fact, this pattern is quite common among 174 genes, indicating that both cis and trans acting gene expression regulation exists, but neither has a 175 strong enough effect to be detected via linear modeling (Fig. S4). 176 177 7) Multiple testing correction methodology: 178 Gene-by-gene statistical analysis such as the linear modeling presented here conducted many 179 independent tests, and therefore required multiple testing corrections to maintain an acceptable 180 experiment-wise false discovery rate. Here, we used the R package *avalue* (Storey 2002) to 181 transform p-values into q-values that account for the distribution of p-values and the number of 182 tests. 183 184 For each set of *p*-values, we removed any NA's, then calculated the *q*-value distribution as well 185 as the estimated proportion of true null hypotheses (π_0), which was estimated with the "bootstrap" 186 method (Storey et al. 2004). 187 188 8) Promoter motif enrichment analytical pipeline: 189 A set of 485 annotated and published plant promoter motifs were downloaded from the 190 newPLACE (Higo et al. 1999) database (http://www.dna.affrc.go.jp/PLACE/) on 9-December 191 2015. These motif sequences were converted into probability matrices via a custom algorithm and 192 read into the R package PWMEnrich (Frith et al. 2004). 193 194 Promoter sequences from all *P. hallii* gene models were downloaded from the Phytozome server 195 (http://phytozome.jgi.doe.gov/pz/portal.html) on 10-December 2015. We developed the 196 background distribution of motifs on a random subset of 2000 promoter sequences using the 197 function makePWMLognBackground. This background distribution was used to generate p-198 values for all motif analyses. We conduced promoter motif enrichment analyses on 7 subsets of 199 genes. For each set of genes, we conducted motif enrichment analyses in the PWMEnrich 200 package using the following parameters: scoring via the "affinity" method and background 201 correction through the *logn* method. P-values were transformed to q-values using the R package

- 202 qvalue (Storey 2002).
- 203

204	
205	II: COMPARISON OF CIS-TRANS TEST METHODOLOGIES
206	
207	1) Comparison to traditional binomial tests:
207	We implemented the methods of Wittkopp et al. (2004), who utilized a Mann Whitney t test to
200	guartify the effects of aig and trans variation using the D has function wildow test. More recently
209	quality the effects of cis and trains variation using the K base function witcox.test. More fecentry,
210	the cis-trans test has also been conducted using exact binomial tests, which we implemented
211	using the R base function <i>binom.test</i> . <i>P</i> -value ranks for these two tests and our negative binomial
212	linear model are presented in Figure S4.
213	
214	2) Comparison of modeling in DESeq2 and LIMMA:
215	An important consideration is that the HAL2 and FIL2 alleles in the F1 plants are not
216	independent. While normalization in DESeq2 accounts for differential library size, other factors,
217	such as the specific attributes of the plant, soil characteristics, pests etc. may cause correlations
218	between the alleles in the F_1 plants. As such, it may be appropriate to account for such
219	correlations using a mixed effects model where the ID of each plant (Plant ID) is a blocking
220	factor. Of particular concern would be any case where the estimated effects of a gene are biased
221	in the DESeq2 approach, but are not significant when plant ID is accounted for.
222	
223	LIMMA is a differential expression analysis package originally designed for microarray
224	experiments. We originally opted for DESeq2 over LIMMA for several reasons: 1) DESeq2 is
225	designed explicitly for RNA-seq datasets (Love et al. 2014), 2) it good FDR control when many
226	biological replicates are present (Soneson and Delorenzi 2013), 3) there is precedent for the
227	analysis of cis and trans factors in DESeq2 (Bader et al. 2015). However, DESeq2 does not offer
228	mixed effects modeling, a drawback that makes it impossible to fit plant ID as a term in the
229	model LIMMA does permit fitting a model with a random a blocking factor
230	However, LIMMA has several drawbacks that make us hesitant to exploit this approach. First
231	LIMMA fits a common correlation among all levels of random effects. Since our blocking factors
232	occur across diverse sampling times and experimental treatments, it is not clear if this approach is
232	appropriate Second LIMMA only permits a single blocking factor, whereas here, we would
233	prefer to fit both time of sampling and unique plant ID as random effects. Finally, the blocking
234	affect in LIMMA was originally designed for technical rankiestes (e.g. multiple arrays). It is not
235	alear how this approach may perform using a blocking factor, like plant ID with 88 levels
230	clear now this approach may perform using a blocking factor, like plant iD with 88 levels.
237	We first compared identical models in DECar2 and LIMMA (Fig. 80). Clearly the results are
230	we first compared identical models in DESeq2 and LIWIWA (Fig. 59). Clearly the results are
239	similar, but DESeq2 otters significantly greater power for the estimation of all model contrasts.
240	2) Commentary of the delivery of the set of
241	5) Comparison of modeling with and without Plant ID as a blocking factor:
242	To test for the significance of the effect of blocking by plant ID, we implemented our model in
243	LIMMA, accounting for the correlation due to the unique plant, and using plant ID as a blocking
244	factor. We then merged these results with the DESeq2 results and plotted the resultant data. It is
245	clear that the LIMMA approach dramatically improves power to detect both cis and trans effects
246	(Fig S10); however, it is not clear if this more complex model accurately fits the data.
247	
248	To assess this, we looked at the residual variance of the two models in LIMMA. Combined, the
249	residual variance was reduced by 1.6% (median) in the model with plantID as a blocking effect.
250	We conclude that the difference between models is very modest difference. Due to its increased
251	power and simplicity, we opted for the DESeq2 fixed model over either of the LIMMA models.
252	
253	
254	

III: SUPPLEMENTARY FIGURES





Figure S1. Climatic context of 2013 drought. The native habitats of HAL2 and FIL2 are

separated by 280km, but exhibit markedly different historical climates during the study period, as
indicated by the amount of precipitation in the warmest quarter. The density distributions of mean
daily precipitation (a) and mean maximum temperature (b) for each year from 1946-2014 are

plotted for each site. The point estimate of the 2013 drought intensity relative to each site isplotted as a vertical arrow

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270 Type of heterosis 271 Figure S3: Distribution of heterosis effects across differential expression and GxE

categories. Panel a presents heterosis categorization of conserved genes, while panels b-e depict
the heterosis categorizations in the four color categories in Fig. 2b-c. The total number of genes in
each category are presented and colored by the treatment: black bars indicate the number of genes
in each heterosis bin from the drought treatment, while white filled bars represent the expression
categorization from the recovery treatment.



Figure S4. Comparison between linear modeling and traditional cis-trans test analyses.

Comparison of the inference of cis (a-c) and trans (d-f) between DESeq2 models ("des cis", "des trans"), mann-whitney test ("Wilcox cis", "Wilcox trans") and exact binomial tests ("binomial cis", "binomial trans"). R² values from a linear model implemented in the R base function "lm" accompany the title of each comparison. All tests are completed using only the dry treatment data.

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comparisons of effects among models



comparisons of effects among models





- 292 ASE (panel b).
- 293





296 Figure S6. Counts and reaction norms of cis*treatment interactions. The values plotted are 297 the quantile normalized least square means of the expression values for each combination of 298 treatment and cis effect for all genes with significant cis*treatment interactions. Genes with 299 significant treatment * cis interactions are broken out into the 14 possible expression patterns. 300 The alleles are colored as FIL2 (green) and HAL2 (black). Accompanying the line plots are bar 301 plots indicating membership in each expression pattern type. Bars from left to right represent 302 membership of each pattern from top to bottom in each column. Column 1 (red barplot) shows all 303 genes where the slopes of each allele are in the same direction, but the relative ranks of the alleles 304 do not change. Column 2 (green barplot) is reserved for genes where the slopes are in the same 305 direction, but the allelic LSmeans change ranks. Column 3 (blue barplot) genes have opposite 306 slopes but retain the same ranks, while column 4 (cyan barplot) genes have both opposite slopes 307 and different ranks.





308 309 Figure S7. Counts and reaction norms of trans effects. The values plotted are the quantile 310 normalized least square means of the expression values for each combination of allele and 311 generation (trans) effect for all genes with significant trans effects. Genes with significant trans 312 effects are broken out into the 14 possible expression patterns. The alleles are colored as FIL2 313 (green) and HAL2 (black). Accompanying the line plots are bar plots indicating membership in 314 each expression pattern type. Bars from left to right represent membership of each pattern from 315 top to bottom in each column. Column 1 (red barplot) shows all genes where the slopes of each 316 allele are in the same direction, but the relative ranks of the alleles do not change. Column 2 317 (green barplot) is reserved for genes where the slopes are in the same direction, but the allelic 318 LSmeans change ranks. Column 3 (blue barplot) genes have opposite slopes but retain the same 319 ranks, while column 4 (cyan barplot) genes have both opposite slopes and different ranks. 320



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Figure S8. Reaction norms across the 9 genes significantly affected by trans*treatment

interactions. Mean log2 transformed, variance stabilized and library size corrected counts are
 plotted for all genes with significant trans*trt effects. The Arabidopsis ortholog TAIR gene
 identifiers and pseudonyms for each gene can be found in Table 2.

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334 Figure S9. Comparison between LIMMA and DESeq2 models for the estimation of cis,

335trans and their interactions with treatment. P-values from each model are plotted on linear336scales. The 1:1 line is plotted as the dashed black line. A gam (formula: $y \sim s(x, bs = "cs")$)337smoothing curve is overlaid to demonstrate where the density of points lies. Points are colored by338the method that determined significance. If neither model found significance, the points are grey.339The number of genes in each category are reported in the legend.



((fit\$sigma - fit2\$sigma)/fit2\$sigma) * 100

Figure S10: Comparisons of a fixed effects model in LIMMA to one with PlantID as a blocking
factor. There is a general increase in the residual variance for the fixed effects model where the
residual variance is very high, but the two models perform equally well with models with lower
residual variance (a-b). Panel c displays a histogram of the y-axis in panel b, marking the mean

(red) and median (blue) change in the percent decrease in residual variance from the fixed effects
model to that with plantID as a blocking factor.

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365	IV: SUPPLEMENTARY TABLE CAPTIONS
366	
367	Table S1. Significant GO terms for five categories of genes: 1) drought-responsive. 2) up-
368	regulated in drought 3) down-regulated in drought 4) cis-by-treatment interaction affects
369	expression 5) trans regulated EDR-corrected P-values are included along with GO id numbers
370	and descriptions of each GO term. Terms are ordered by significance
271	and descriptions of each of term. Terms are ordered by significance.
371	Table C2. Cianificantly and the langement of the terms for 7 action of a second All matrice
372	Table S2: Significantly enriched promoter motifs terms for / categories of genes. All motifs
373	passing an $\alpha = 0.05$ threshold are reported. The annotations and references, parsed from the
374	newPLACE database, accompany the motifs. The column "TEST" corresponds to the subset of
375	genes analyzed: GxE genes with crossing reaction norms (green points, fig 2a) that were either up
376	regulated ("gxe.halup.fildown") or down regulated in HAL2 in drought ("gxe.haldown.filup"), 3)
377	Genes with very significant trans effects ("all trans q<0.01"), 4) genes with significant signatures
378	of compensatory evolution ("compensatory"), 5) genes with cis-by-treatment interactions
379	("cis.trt").
380	
381	Column names: ID = motif ID (newPLACE) $RANK$ = a value derived rank of each motif for
382	each test $RAW SCORE$ = the affinity test statistic score. $PVALUE$ = background corrected <i>n</i> -
383	value for the affinity test $TFST =$ the group of genes used to make the test (described above) O
384	$VALUE = q_{value}$ calculated from the complete p_{value} distribution SEQUENCE = The motif
205	$\gamma AEOE = q$ -value calculated from the complete p-value distribution, $SEQOENCE =$ The motification $\gamma ACCESSION# =$ the new DLACE ecoession numeric identifier. DESCRIPTION = the
202	sequence, ACCESSION# – the new PLACE accession numeric identifier, DESCRIFTION – the
207	newPLACE description, ASSOCIATED - newPLACE associated characteristics, ORGANISM -
30/	newPLACE organism in which the motif was identified, <i>REFERINCE</i> = newPLACE databased
388	references that documented said motif.
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391	V: SUPPLEMENTARY REFERENCES
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