1 **Title: Genotype-by-Environment Interactions Affecting Heterosis in Maize**

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ABSTRACT

The environment can influence heterosis, the phenomena in which the offspring of two inbred parents exhibits phenotypic performance beyond the inbred parents for specific traits. In this study we measured 25 traits in a set of 47 maize hybrids and their inbred parents grown in 16 different environments, and each had varying levels of average productivity. By quantifying 25 vegetative and reproductive traits across the life cycle we were able to analyze interactions between the environment and multiple distinct instances of heterosis. The magnitude and rank among hybrids of better-parent heterosis (BPH) varied for the different traits and environments. Across the traits, a higher within plot variance was observed for inbred lines compared to hybrids. However, for most traits, variance across environments was not significantly different for inbred lines compared to hybrids. Further, for many traits the correlations of BPH to hybrid performance and BPH to better parent performance were of comparable magnitude. These results indicate that inbreds and hybrids are showing similar trends in environmental response and are both contribute to genotype-by-environment interactions for heterosis. This study highlights that degree of heterosis is not an inherent trait of a specific hybrid, but varies depending on the trait measured and the environment where that trait is measured. Studies that attempt to correlate molecular processes with heterosis are hindered by the fact that heterosis is not a consistent attribute of a specific hybrid.

INTRODUCTION

Heterosis, or hybrid vigor, refers to the phenomena in which the offspring of two inbred parents exhibits phenotypic performance beyond the mid-parent or best parent used to generate the hybrid. Heterosis has been observed in many plant and animal species (Janick 1998; Melchinger and Gumber 1998). Notably, the heterosis of mules (the ability to perform more work with fewer resources) was widely utilized in agriculture prior to mechanization (Troyer 2006). Inbreeding depression and heterosis in maize was initially documented by George H. Shull and Edward M. East (East 1908; Shull 1908; Shull 1909). The adoption of hybrid maize over open-pollinated varieties occurred remarkably fast due to improved yields, greater uniformity for machine harvesting, and increased durability under extreme abiotic stress. In just a four-year period of time, hybrid maize acreage went from less than 10% to over 90% in Iowa (Crow 1998). The widespread utilization of heterosis now shapes breeding programs for several agriculturally important species including maize and rice.

There is widespread interest in developing methods to characterize the molecular basis of heterosis, and to predict hybrid performance to increase the efficiency of hybrid breeding programs. Researchers have attempted to utilize genomic sequence (Riedelsheimer et al. 2012), RNA expression levels of genes (Melchinger and Gumber 1998; Frisch et al. 2010; Scholten and Thiemann 2013), sRNAs (Groszmann et al. 2011; Zhang et al. 2014), proteomic (Dahal et al. 2012), and metabolomic (Riedelsheimer et al. 2012; de Abreu et al. 2017) data to predict or dissect heterosis (Schnable and Springer 2013). While relationships have been identified using each of these data types, no data type is able to completely predict hybrid performance individually (Kaeppler 2012). Attempts to predict hybrid performance are complicated by the

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87 fact that heterosis levels vary for different traits within the same hybrid (Flint-Garcia et al.

88 2009).

89   Although plant breeders have noticed that hybrid genotypes are more stress tolerant than

90 their inbred parents, there are few published reports to support this conclusion, particularly in

91 environments with moderate rather than extreme levels of abiotic stress. In *Arabidopsis*, stress

92 response gene expression networks have been shown to contribute to heterosis and the

93 prediction of hybrid performance (Groszmann et al. 2015; Miller et al. 2015). While variation in

94 levels of heterosis have been observed under different growing conditions, there are few

95 studies that document changes in heterosis across diverse environmental conditions and traits.

96   In this study we measured 25 traits in 47 maize hybrids and their inbred parents that were

97 grown in 16 different environments. The objective of the study was to document variation in

98 estimates of heterosis across traits and environments. The results provide evidence that

99 unravelling the molecular basis of heterosis is challenging because heterosis is not a fixed

100 attribute of an individual across space, time, or environmental conditions.

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102 **MATERIALS AND METHODS**

103 **Germplasm**

104 Eleven inbred lines were selected representing important founders in commercial maize

105 breeding programs including DK3IIH6 (PI 564754), LH145 (PI 600959), LH185 (PI 576171), LH198

106 (PI 557563), LH82 (PI 601170), PHB47 (PI 601009), PHK56 (PI 543842), PHK76 (PI 601496),

107 PHN46 (PI 543844), PHP02 (PI 601570), and a recent release W606S. These inbred lines

108 represent multiple heterotic groups including Iodent (DK3IIH6, PHP02), Non-Stiff Stalk (LH185,

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109   LH82, PHK76, PHN46, PHK56, W606S), and Stiff Stalk (LH145, LH198, PHB47). These lines, with

110   the exception of W606S, are all commercial inbred lines that have expired Plant Variety

111   Protection certificates, and thus represent elite maize germplasm. These inbred lines were

112   crossed in a partial diallel to generate 47 hybrid genotypes (see Table S1).

113   To evaluate genetic diversity between the parental lines used to generate the hybrid

114   genotypes, genetic similarity between the parents was calculated using whole genome identity

115   by state (Purcell et al. 2007) using 430,000 SNPs derived from RNA-sequencing (Hirsch et al.

116   2014).

117

118   **Field Evaluations**

119   Trials containing single row plots (3.35 m long and 0.76 m apart) were planted in a total of 16

120   environments in Iowa, Minnesota, and Wisconsin in the summer of 2015. The 16 environments

121   were defined by location (5 separate locations), and management practices within location

122   (planting date; high (70,000 plants ha^{-1}) and low (20,000 plants ha^{-1}) plant density). Arlington,

123   WI and Waseca, MN had high and low planting densities, representing a total of four

124   environments. Curtiss, IA, Kelly, IA, and St. Paul, MN had a factorial of high and low planting

125   density and early and late planting at each site, representing a total of 12 environments (see

126   Table S2). Within each location/management environment there were two replications and

127   hybrids were blocked separately from inbred lines within each replication.

128   Twelve vegetative traits were measured on six representative plants per plot. These traits

129   included plant height at 14, 21, 28, 35, 42, 49, 56, and 63 days after planting (DAP) measured as

130   the distance from the soil surface to the uppermost leaf tip when the leaves were pulled

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131   upright. Plant height at maturity was measured from the soil surface to the collar of the flag

132   leaf, ear height at maturity was measured from the soil surface to the node subtending the

133   uppermost ear. Leaf number above the ear, and leaf number (including senesced leaves) below

134   the ear were counted after anthesis. Juvenile leaves were marked to allow leaf number

135   including senesced leaves to be counted using previously described methods (Hirsch et al.

136   2014). Days to anthesis and days to silk were measured on a per-plot basis as the day on which

137   approximately half of the plants in the plot were shedding pollen and the day on which half of

138   the plants in the plot had exposed silks, respectively. Custom computer algorithms executed on

139   Open Science Grid computational resources (Pordes et al. 2007) in a workflow managed by

140   HTCondor software (Thain et al. 2005) quantified eleven ear and kernel traits from digital

141   images as previously described (Miller et al. 2017). Six representative ears per plot were

142   measured. Ear weight and grain weight was an average of the weight of the uppermost ear on

143   the six representative plants in the plot and cob weight was measured on individual uppermost

144   ears from the six representative plants in the plot (Table 1 and see Table S3). For all traits for

145   which single plant measurements were taken, the same six representative plants were used for

146   all measurements. See Table S2 for details on which traits were measured in each environment

147   and Table S3 for raw phenotypic values.

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149   **Statistical Analyses**

150   Better-parent heterosis (BPH) and percent better-parent heterosis (%BPH) were calculated for

151   each trait and hybrid within each replicate block as BPH = hybrid phenotype - better-parent

152   phenotype and %BPH = ((hybrid phenotype - better-parent phenotype)/Better-parent

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153 phenotype) x 100, respectively and then averaged across replicates within an environment. The

154 average %BPH of the two replicates in each environment was used for subsequent analyses. For

155 all traits except flowering time the higher parent was considered the better parent. For

156 flowering time, the earlier parent was considered the better parent.

157 A mixed model analysis was performed using PROC GLM in SAS 9.0 (SAS Institute 2002) to

158 partition variation into genotype, environment, genotype-by-environment interaction, and

159 error variances for each trait with all sources of variation considered random. This analysis was

160 done for inbred traits per se, hybrid traits per se, and heterosis for all 25 traits. Pearson

161 correlation coefficients and corresponding significant tests was conducted using PROC CORR in

162 SAS 9.0 (SAS Institute 2002). A mixed linear model was constructed by PROC MIXED in SAS 9.0

163 (SAS Institute 2002) to get the best linear unbiased prediction for each hybrid and inbred across

164 the 16 environments: $y_i = \mu + f_i + e_i + \varepsilon_i$, where y_i is phenotypic value of individual i, μ is the

165 phenotypic mean of multiple environments, f_i is genotype effect, e_i is environmental effect,

166 and ε_i is the residual effect. All the variables except μ were considered as random effects

167 (Bernardo 1994, 1996; Henderson 1975, 1984). The coefficient of variation (CV) for traits was

168 calculated as the standard deviation divided by the plot mean. This is the most widely used

169 parameter to quantify variability of traits with different units of measurement among individual

170 plants and across environments (Munaro et al. 2011a).

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172 **Statement on data and reagent availability**

173 All raw phenotypic data is available in Supplemental Table 3 and GPS coordinates of locations

174 and growth conditions are available in Supplemental Table 2.

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176 **RESULTS AND DISCUSSION**

177 **Better parent heterosis is variable across traits, environments, and developmental time**

178 The majority of 25 measured traits exhibited significant genotype, environmental, and

179 genotype-by-environment interaction effects in both the inbred lines and the hybrids across the

180 16 environments as well as for BPH (see Table S4). Better-parent heterosis (BPH) was detected

181 for most of the 25 traits, and 16 of them exhibited BPH in more than 90% of hybrids (Table 1).

182 Only two traits, leaf number below the ear (LNB) and kernel depth (KD), exhibited BPH in fewer

183 than 50% of the hybrids. The average BPH varied substantially among the different traits (Table

184 1). Some traits such as grain yield per plant (GWT) exhibited BPH values greater than 90% BPH

185 while other traits such as flowering time (DTA/DTS) exhibited a lower magnitude of BPH (-4.4 to

186 -5.0%). However, for both of these traits the majority of hybrids exhibited BPH across all

187 environments (Table 1).

188 The correlation of BPH across the traits studied varied substantially ranging from (r=-0.33

189 for EWT and PHt63 to 0.99 for EWT BPH and GWT BPH; Figure 1A and 1B), similar to previous

190 observations (Flint-Garcia et al. 2009). A network visualization of the correlations between BPH

191 for distinct traits revealed several trends (Figure 1B). Strong positive correlations were

192 observed within groups of traits that likely share a common genetic, physiological and

193 developmental basis, including yield related traits (cob, ear, and kernel traits) and vegetative

194 traits including plant height at 14DAP through maturity and ear height (Figure 1A and 1B). Days

195 to anthesis (DTS) BPH and plant height 63 days after planting (PHt63) BPH had strong negative

196 correlations with grain weight (GWT) BPH, ear weight (EWT) BPH and ear width (EW) BPH

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197   because the better parent was the one that flowered early, and the hybrids flowered generally

198   earlier than the better parent. PHt63 BPH was correlated with both vegetative and reproductive

199   plant traits, connecting the two subgroups (Figure 1B).

200   The 47 hybrids could be assigned into three general clusters based on their relative

201   heterosis performance (rank number) across the 25 traits (Figure 1C). The first cluster (n=7)

202   exhibited consistently lower BPH for all the traits relative to other hybrids and was significantly

203   enriched (Table S5) for within heterotic group hybrids (NSS x NSS). Hybrids in the second cluster

204   (n=18) showed relatively high heterosis for yield-related and flowering time traits, but lower

205   heterosis for most of the vegetative traits, while hybrids in the third cluster (n=22) were the

206   opposite (Figure 1C).

207   Among the 47 hybrids genotypes, identity by state values ranged from 0.67 to 0.86 for the

208   widest and narrowest crosses. Genotype clusters 1, 2, and 3 had identity by state averages of

209   0.755, 0.694, and 0.702 respectively. The hybrid genotypes in cluster 1, which had the lowest

210   relative heterosis across all traits, was composed of relatively narrow crosses. The hybrid

211   genotypes in cluster 2, which had the highest amount of heterosis across yield-related and

212   flowering traits, was composed of relatively wide SSS x NSS crosses. This supports the historical

213   convention of breeders crossing between heterotic pools of unrelated inbreds to maximize

214   heterosis for yield related traits.

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216   **There is low predictive capacity of heterosis over developmental time**

217   It is desirable to identify traits early in development that predict heterosis and yield at the end

218   of the season. Previous reports indicate that traits measured at maturity showed the highest

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219   level of heterosis (Falconer and Mackay 1996). However, it has been shown that heterosis could

220   already be detected during early stages of maize seedling growth (Hoecker et al. 2006) and

221   embryo development (Paschold et al. 2010; Meyer et al. 2007).

222   To determine the potential of heterosis based on early developmental stages to predict

223   heterosis at later development stages we measured heterosis for plant height at seven

224   developmental stages ranging from 14 days after planting to anthesis. A low correlation was

225   observed between heterosis for plant height at early developmental stages and at anthesis

226   (Figure 2). However, final plant height was more highly correlated with measures at

227   developmental stages closer to anthesis. Overall, our results indicate that heterosis measured

228   at the seedling stage is not predictive of heterosis at the adult stage.

229   These low levels of correlation could potentially be a product of low correlation for the

230   hybrid performance, the better parent performance, or both. To evaluate what drives this

231   reduced correlation in heterosis over increased windows of developmental time, correlation

232   coefficients for hybrid performance over time and inbred performance over time were overlaid

233   with heterosis correlations (Figure 2). Both hybrid performance and inbred performance

234   showed a similar tendency over time, indicating that both hybrid and better parent

235   performance have a comparable effect on the lack of correlation from early stages of

236   development to maturity. Given the inability to predict heterosis levels, or even heterosis ranks,

237   for the same trait (plant height) collected at different stages of development it is likely to be

238   quite difficult to predict adult plant traits from seedling traits or to relate specific heterosis

239   mechanisms observed in the seedling to those contributing to variation in heterosis for traits at

240   maturity.

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242   **Performance of hybrids is more stable than inbred lines within but not among environments**

243   Differential responses of maize hybrids and/or inbred lines to environmental stimuli will result

244   in altered levels of heterosis across environments (Munaro et al. 2011b; Munaro et al. 2011a).

245   Evidence from multiple species indicates that hybrids performance is more stable across

246   environments than inbred performance (Cole et al. 2009). This observation is consistent with

247   the concept of "buffering" in which heterogeneous populations or heterozygous individuals are

248   more stable than homogeneous populations or homozygous individuals (Allard and Bradshaw

249   1964; Schnell and Becker 1986; Cole et al. 2009). We compared the stability of inbred and

250   hybrid traits both within an environment and among environments.

251   To evaluate stability across traits the coefficient of variation (CV) was used. The within plot

252   CV for inbred lines in this study was greater than the within plot CV for hybrids for nearly every

253   trait measured (Figure 3A), providing evidence for greater variability of inbred lines within

254   environments for most traits. We also assessed the CV among environments for each trait in

255   the inbred and hybrid lines (Figure 3B). For ten of the traits the inbred lines exhibited a

256   significantly higher CV than the hybrids, indicating that for these traits instability across

257   environments was driven more by the instability of inbred lines. However, for flowering time

258   traits (DTA and DTS), hybrids had significantly higher CV than inbred lines across the

259   environments. The remaining 13 traits did not exhibit significant differences between the

260   hybrid and inbred lines for the CV among environments. For the plant height measurements

261   over developmental time, the CV among environments decreased throughout time for both

262   inbred lines and hybrids, indicating increasing stability of both hybrids and inbred lines at later

263   developmental stages.

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265   **Factors influencing environmental variation for heterosis are variable across traits**

266   We were interested in assessing the factors contributing to the significant genotype-by-

267   environment interaction effect on heterosis for most of the traits in this study. We focused on

268   grain weight (GWT) and plant height at maturity (PHt). These traits have variable heritability,

269   and BPH for these traits were not significantly correlated across genotypes or environments

270   (Figure 1).

271   There were differences in the patterns of BPH among environments observed for GWT and

272   PHt (Figure 4A and 4C). GWT generally expressed a greater BPH in low planting density

273   environments, while planting density seemed to have little impact on BPH of PHt. For GWT, the

274   correlation of IBS and BPH at high density was slightly higher (r=-0.58***) than for low density

275   (r=-0.52***) indicating that BPH may be more affected by IBS at high density environments.

276   However, both are highly correlated and IBS is affecting BPH under both conditions. For each of

277   the traits the stability and average BPH was quite variable among hybrids (Figure 4). The hybrid

278   that expressed the lowest and highest BPH based on BLUP values across all the environments

279   were identified for each trait (indicated by arrows in Figure 4A and 4C). The stability of

280   heterosis in these hybrids was evaluated across environments. Interestingly, for PHt the hybrid

281   with the highest BPH exhibited consistently high levels of BPH while the hybrid with the lowest

282   average BPH exhibited quite variable heterosis among environments (Figure 4D). However, this

283   hybrid also had lower hybrid performance and therefore this result may be due to sensitivity to

13

284   variable neighbor effects. The opposite pattern was observed for the hybrids with highest and

285   lowest average BPH for GWT (Figure 4C). This trend was consistent across the entire set of 47

286   hybrids (see Figure S1). This may suggest that hybrids with the highest potential for GWT are

287   the most responsive and have the potential to take advantage of favorable environments.

288   BPH is a measure of the difference in performance of the hybrid relative to the parents.

289   Environmental influences on BPH could reflect changes in hybrid performance, changes in

290   inbred performance or a combination of both. We investigated the patterns of BPH, hybrid

291   performance and inbred performance for GWT and PHt in a selected set of hybrids (Figure 5).

292   We first assessed the patterns for the hybrids with the highest average BPH for GWT (Figure 5A)

293   and PHt (Figure 5E). We also assessed the patterns for the hybrid with the greatest (Figure 5B

294   and 5F) or least (Figure 5C and 5G) standard deviation for BPH ranks among the environments.

295   These reveal a variety of patterns in the trend of inbred and hybrid performance relative to

296   variation in BPH values among environments. There are examples, such as Figure 5E, in which

297   the reduction in heterosis in some environments is due to reduced hybrid performance with

298   relatively stable inbred performance. In other examples, such as Figure 5C, the changes in

299   heterosis seem to be driven by changes in the inbred performance among the environments.

300   We proceeded to assess the relative contribution of variation in the inbreds and hybrids to

301   the environmental variation for BPH for all 47 hybrids for GWT and PHt (Figure 5D and 5H).

302   The correlation of better parent performance and BPH (y-axis) was plotted against the

303   correlation of hybrid and BPH (x-axis). As expected, the performance of the better parent tends

304   to be negatively correlated with heterosis while the performance of the hybrid is positively

305   correlated with heterosis. If variation for better parent performance and hybrid performance

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306   equally contribute to heterosis variation we would expect a slope of one in the regression line

307   of this plot. The observed slope was less than one, indicating that variation in the hybrids was

308   contributing slightly more to the observed BPH values than variation in the inbred lines. There

309   are differences in the distribution of the correlation values for GWT (Figure 5D) and PHt (Figure

310   5H). For GWT, 46 of 47 hybrids have a positive correlation between hybrid performance and

311   heterosis (Figure 5D) suggesting that heterosis for GWT is influenced by hybrid performance in

312   all genotypes.   In contrast, there are a number of hybrids without significant correlations

313   between hybrid performance and heterosis for PHt (Figure 5H).

314   We assessed the relative influence of better parent and hybrid variation on BPH for all 25

315   traits measured in this study (Table S6). In the majority of cases the hybrid performance is

316   positively correlated with heterosis while the better parent performance is negatively

317   correlated with heterosis. However, the relative strength of the correlations varied among

318   different traits. For traits such as KD, PHt, DW there was a much stronger correlation between

319   better parent performance and heterosis. Environmental variation for heterosis for other traits

320   such as CWT, KW, and EL are more strongly influenced by the hybrid performance (Table S6).

321   Interestingly, GWT showed equal strength of correlation for both hybrid performance with

322   heterosis and better parent performance with heterosis. There was, however, a significant

323   negative correlation between "noise" (residual from ANOVA using BPH) and the correlation of

324   better parent performance and BPH (r=-0.77***), which may impact the ability to accurately

325   assess the relative contribution of inbreds and hybrids to observed BPH.

326   Corn yields have increased continuously since hybrids were first commercially grown in the

327   1930s. However, the increase in yield of commercial hybrids has not been attributed to an

328   increase in heterosis (Fasoula and Fasoula 2005). Indeed, the percentage of heterosis has not

329   changed substantially, and by some estimates has decreased slightly over time due to the

330   higher percentage rate of gain in yield for inbred lines relative to hybrids (Duvick 1999; Troyer

331   and Wellin 2009). Our data suggest that variation in the performance of inbred lines and hybrid

332   lines in different environments will influence the amount of heterosis. The relative influence of

333   hybrid variation and inbred variation on heterosis is variable across the traits that were

334   measured in this study. It is worth noting that in some extreme environments inbred lines may

335   be severely affected while hybrids are not, and this outcome will influence measures heterosis

336   (Griffing and Zsiros 1971). However, in the moderate environments surveyed in this study we

337   find important contributions of both hybrid and inbred performance to heterosis variation.

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345   **LITERATURE CITED**

346   Allard, R.W., and A.D. Bradshaw, 1964 Implications of Genotype-Environmental Interactions in

347   Applied Plant Breeding1. *Crop Science* 4 (5):503-508.

348   Bernardo, R., 1994 Prediction of Maize Single-Cross Performance Using RFLPs and Information

349   from Related Hybrids. *Crop Science* 34 (1):20-25.

350   Bernardo, R., 1996 Best Linear Unbiased Prediction of Maize Single-Cross Performance. *Crop*

351   *Science* 36 (1):50-56.

352   Cole, C.B., D.T. Bowman, F.M. Bourland, W.D. Caldwell, B.T. Campbell *et al.*, 2009 Impact of

353   Heterozygosity and Heterogeneity on Cotton Lint Yield Stability All rights reserved. No part

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357   reprinting the material contained herein has been obtained by the publisher. *Crop Science*

358   49 (5):1577-1585.

359   Crow, J.F., 1998 90 years ago: the beginning of hybrid maize. *Genetics* 148 (3):923-928.

360   Dahal, D., B.P. Mooney, and K.J. Newton, 2012 Specific changes in total and mitochondrial

361   proteomes are associated with higher levels of heterosis in maize hybrids. *Plant J* 72 (1):70-

362   83.

363   de Abreu, E.L.F., M. Westhues, A. Cuadros-Inostroza, L. Willmitzer, A.E. Melchinger *et al.*, 2017

364   Metabolic robustness in young roots underpins a predictive model of maize hybrid

365   performance in the field. *Plant J.*

366   Duvick, D.N., 1999 Heterosis: Feeding People and Protecting Natural Resources, pp. 19-29 in

367   *The Genetics and Exploitation of Heterosis in Crops*, edited by J.G. Coors and S. Pandey.

368   American Society of Agronomy, Crop Science Society of America, Madison, WI.

369   East, E.M., 1908 Inbreeding in corn. *Conn. Agric. Exp. Stn. Rep.* (419-428).

370   Falconer, D., and T. Mackay, 1996 *Introduction to Quantitative Genetics (4th Edition)*.

371   Fasoula, D.A., and V.A. Fasoula, 2005 Bridging the productivity gap between maize inbreds and

372   hybrids by replacing gene and genome dichotomization with gene and genome. *Maydica*

373   50:49-61.

374   Flint-Garcia, S.A., E.S. Buckler, P. Tiffin, E. Ersoz, and N.M. Springer, 2009 Heterosis is prevalent

375   for multiple traits in diverse maize germplasm. *PLoS One* 4 (10):e7433.

376   Frisch, M., A. Thiemann, J. Fu, T.A. Schrag, S. Scholten *et al.*, 2010 Transcriptome-based distance

377   measures for grouping of germplasm and prediction of hybrid performance in maize.

378   *Theoretical and Applied Genetics* 120 (2):441-450.

379   Griffing, B., and E. Zsiros, 1971 Heterosis associated with genotype-environment interactions.

380   *Genetics* 68 (3):443-455.

381   Groszmann, M., R. Gonzalez-Bayon, R.L. Lyons, I.K. Greaves, K. Kazan *et al.*, 2015 Hormone-

382   regulated defense and stress response networks contribute to heterosis in Arabidopsis F1

383   hybrids. *Proc Natl Acad Sci U S A* 112 (46):E6397-6406.

384   Groszmann, M., I.K. Greaves, Z.I. Albertyn, G.N. Scofield, W.J. Peacock *et al.*, 2011 Changes in

385   24-nt siRNA levels in Arabidopsis hybrids suggest an epigenetic contribution to hybrid vigor.

386   *Proc Natl Acad Sci U S A* 108 (6):2617-2622.

387   Henderson, C.R., 1975 Best linear unbiased estimation and prediction under a selection model.

388   *Biometrics* 31 (2):423-447.

389   Henderson, C.R., 1984 *Applications of Linear Models in Animal Breeding*: Guelph, Ont:

390   University of Guelph.

391   Hirsch, C.N., J.M. Foerster, J.M. Johnson, R.S. Sekhon, G. Muttoni *et al.*, 2014 Insights into the

392   maize pan-genome and pan-transcriptome. *Plant Cell* 26 (1):121-135.

393   Hoecker, N., B. Keller, H.P. Piepho, and F. Hochholdinger, 2006 Manifestation of heterosis

394   during early maize (Zea mays L.) root development. *Theor Appl Genet* 112 (3):421-429.

395   Janick, J., 1998 Hybrids in horticultural crops. *Concepts and breeding of heterosis in crop plants*

396   (conceptsandbree):45-56.

397   Kaeppler, S., 2012 Heterosis: Many Genes, Many Mechanisms—End the Search for an

398   Undiscovered Unifying Theory. *ISRN Botany* 2012:12.

399   Melchinger, A.E., and R.K. Gumber, 1998 Overview of heterosis and heterotic groups in

400   agronomic crops. *Concepts and breeding of heterosis in crop plants* (conceptsandbree):29-

401   44.

402   Meyer, S., H. Pospisil, and S. Scholten, 2007 Heterosis associated gene expression in maize

403   embryos 6 days after fertilization exhibits additive, dominant and overdominant pattern.

404   *Plant Mol Biol* 63 (3):381-391.

405   Miller, M., Q. Song, X. Shi, T.E. Juenger, and Z.J. Chen, 2015 Natural variation in timing of stress-

406   responsive gene expression predicts heterosis in intraspecific hybrids of Arabidopsis. *Nat*

407   *Commun* 6:7453.

408   Miller, N.D., N.J. Haase, J. Lee, S.M. Kaeppler, N. de Leon *et al.*, 2017 A robust, high-throughput

409   method for computing maize ear, cob, and kernel attributes automatically from images.

410   *Plant J* 89 (1):169-178.

411   Munaro, E.M., K.E. D'Andrea, M.E. Otegui, A.G. Cirilo, and G.H. Eyhérabide, 2011a Heterotic

412   Response for Grain Yield and Ecophysiological Related Traits to Nitrogen Availability in

413   Maize. v. 51.

414   Munaro, E.M., G.H. Eyhérabide, K.E. D'Andrea, A.G. Cirilo, and M.E. Otegui, 2011b Heterosis ×

415   environment interaction in maize: What drives heterosis for grain yield? *Field Crops*

416   *Research* 124 (3):441-449.

417   Paschold, A., C. Marcon, N. Hoecker, and F. Hochholdinger, 2010 Molecular dissection of

418   heterosis manifestation during early maize root development. *Theor Appl Genet* 120

419   (2):383-388.

420   Pordes, R., D. Petravick, B. Kramer, D. Olson, M. Livny *et al.*, 2007 The open science grid. *Journal*

421   *of Physics: Conference Series* 78 (1).

422   Purcell, S., B. Neale, K. Todd-Brown, L. Thomas, M.A. Ferreira *et al.*, 2007 PLINK: a tool set for

423   whole-genome association and population-based linkage analyses. *Am J Hum Genet* 81

424   (3):559-575.

425   Riedelsheimer, C., A. Czedik-Eysenberg, C. Grieder, J. Lisec, F. Technow *et al.*, 2012 Genomic

426   and metabolic prediction of complex heterotic traits in hybrid maize. *Nat Genet* 44 (2):217-

427   220.

428   SAS Institute, 2002 The SAS system for windows version 9.0. *SAS Inst, Cart, NC.*

429   Schnable, P.S., and N.M. Springer, 2013 Progress Toward Understanding Heterosis in Crop

430   Plants. *Annual Review of Plant Biology* 64 (1):71-88.

431   Schnell, F.W., and H.C. Becker, 1986 Yield and Yield Stability in a Balanced System of Widely

432   Differing Population Structures in Zea Mays L. *Plant Breeding* 97 (1):30-38.

433   Scholten, S., and A. Thiemann, 2013 Transcriptome-Based Prediction of Heterosis and Hybrid

434   Performance, pp. 265-279 in *Diagnostics in Plant Breeding*, edited by T. Lübberstedt and

435   R.K. Varshney. Springer Netherlands, Dordrecht.

436   Shull, G.H., 1908 The composition of a field of maize. *Journal of Heredity* os-4 (1):296-301.

437   Shull, G.H., 1909 A pure-line method in corn breeding. *Journal of Heredity* os-5 (1):51-58.

438   Thain, D., T. Tannenbaum, and M. Livny, 2005 Distributed computing in practice: the Condor

439   experience. *Concurrency and Computation: Practice and Experience* 17 (2-4):323-356.

440   Troyer, A.F., 2006 Adaptedness and Heterosis in Corn and Mule Hybrids. *Crop Science* 46

441   (2):528-543.

442   Troyer, A.F., and E.J. Wellin, 2009 Heterosis Decreasing in Hybrids: Yield Test Inbreds All rights

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445   storage and retrieval system, without permission in writing from the publisher. Permission

446   for printing and for reprinting the material contained herein has been obtained by the

447   publisher. *Crop Science* 49 (6):1969-1976.

448   Zhang, L., Y. Peng, X. Wei, Y. Dai, D. Yuan *et al.*, 2014 Small RNAs as important regulators for the

449   hybrid vigour of super-hybrid rice. *J Exp Bot* 65 (20):5989-6002.

450

FIGURE LEGENDS

Figure 1. Better parent heterosis (BPH) comparisons for 25 traits and 47 hybrids across 16 environments. A) Pearson correlation coefficients (r) of BPH between traits; gray shaded cells indicate missing data. B) Network visualization of Pearson correlation coefficients of BPH between traits. Only correlation coefficients less than -0.3 or greater than 0.3 are shown. Traits in yellow circles and green rectangles are reproductive and vegetative traits, respectively. Red line, $r<-0.3$; gray line, $0.3<r<0.5$; blue line, $r>0.5$. C) Average BPH rank scaled with white (highest BPH rank) to dark blue (lowest BPH rank). Hybrid genotypes are followed by the parental identity by state value.

Figure 2. Correlation coefficient for percent better parent heterosis (%BPH), hybrid performance, and better-parent performance of plant height at different development stages in different environments. The numbers of 14-49 in x-axis indicate days after planting and PHt is plant height at physiological maturity.

Figure 3. Coefficient of variation within and across environments for hybrid and inbred genotypes. A) Coefficient of variation within plot (6 plants were phenotyped within each plot). B) Coefficient of variation across all available environments for each trait. In each figure blue and red colors indicate hybrid and inbred, respectively. BLUP values of all available environments for each hybrid and inbred were used. * significant at $p=0.05$; ** significant at $p=0.01$; *** significant at $p=0.001$ in a two-tail t-test between the inbred and hybrid genotypes.

22

473 **Figure 4.** Percent better parent heterosis (%BPH) for grain weight (GWT) and plant height at

474 maturity (PHt) for 47 hybrids across 16 environments. A and C) Heatmap of %BPH for GWT (A)

475 and PHt (C); black shaded cells indicate missing data. The green and blue arrow in each plot

476 indicates the hybrids that have the highest and lowest %BPH across all 16 environments based

477 on BLUP values. Environments and hybrids were clustered using hierarchical clustering (trees

478 not shown). B and D) Highest (indicated by green arrows in A and C) vs. lowest (indicated by

479 blue arrows in A and C) %BPH hybrids across all environments for GWT (B) and PHt (D). Red

480 dots are the eight low-density environments and black dots are the eight high-density

481 environments. H7 is PHP02 x DK3IIH6, H30 is LH185 x DK3IIH6, H32 is LH198 x LH185, H34 is

482 LH82 x W606S.

483

484 **Figure 5.** Relationships among percent better parent heterosis (%BPH), hybrid, and better-

485 parent performance. Plots A-D are for grain weight (GWT) and E-H are for plant height at

486 maturity (PHt). A and E) Hybrids with the highest %BPH across 16 environments. B and F)

487 Hybrids with the highest standard deviation of the rank of %BPH among all 47 entries. C and G)

488 Hybrids with the lowest standard deviation of the rank of %BPH among all 47 entries. D and H)

489 Correlation coefficient of hybrid vs. %BPH and better-parent vs. %BPH (BLUP value across 16

490 environments for each hybrid). Colored dots represent the highest %BPH (red – A and E),

491 highest standard deviation of the rank of %BPH (green – B and F), and lowest standard

492 deviation of the rank of %BPH (blue – C and G). For A-C and E-G dots along the x-axis represent

493 each of the 16 environments.

494

Table 1. Summary of better parent heterosis (BPH) for 25 traits measured across 16 environments

Trait	Abbreviation	Locations	Plots	Average %BPH	% Entries with BPH[a]
Plant Height 14 DAP	PHt14	4	169	20.2	95.3
Plant Height 21 DAP	PHt21	8	340	24.3	95.6
Plant Height 28 DAP	PHt28	12	528	27.9	98.1
Plant Height 35 DAP	PHt35	12	538	30.4	99.4
Plant Height 42 DAP	PHt42	12	538	33.8	99.8
Plant Height 49 DAP	PHt49	10	461	35.8	99.8
Plant Height 56 DAP	PHt56	8	282	38.5	100.0
Plant Height 63 DAP	PHt63	2	94	41.4	100.0
Plant Height Maturity	PHt	16	716	27.9	100.0
Ear Height Maturity	EH	16	716	36.5	99.3
Days to Anthesis[b]	DTA	16	670	-4.4	82.1
Days to Silk[b]	DTS	16	670	-5.0	87.0
Leaf Number Above Ear	LNA	12	538	7.6	53.9
Leaf Number Below Ear	LNB	8	375	3.1	20.0
Cob Width	CW	16	714	8.8	82.1
Cob Length	CL	16	714	20.3	93.1
Cob Weight	CWT	16	714	50.5	91.2
Ear Width	EW	16	710	13.5	95.4
Ear length	EL	16	713	18.6	91.6
Ear Weight	EWT	16	714	84.9	98.9
Kernel Row Number	KRN	16	713	7.2	58.6
Kernel Height	KH	16	710	26.1	89.6
Kernel Width	KW	16	709	8.7	59.4
Kernel Depth	KD	16	714	2.8	1.5
Per Plant Grain Weight	GWT	16	714	91.4	99.4

[a]Entry is the inbred/hybrid combination within an environment
[b]For these traits the better-parent performance was the lower value.
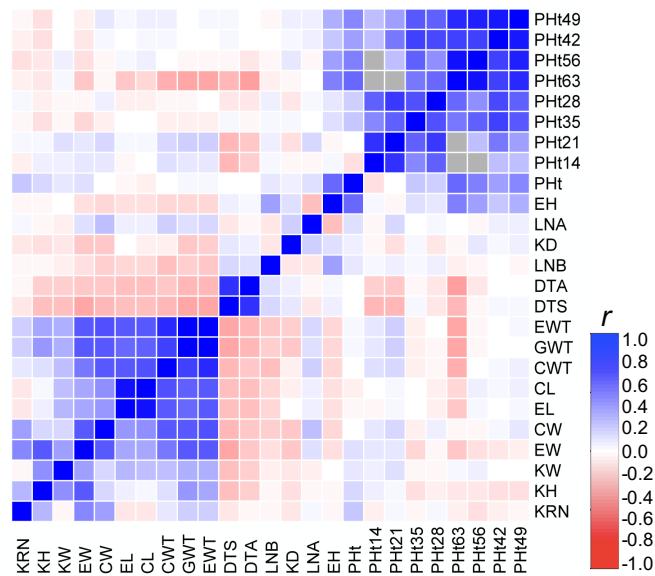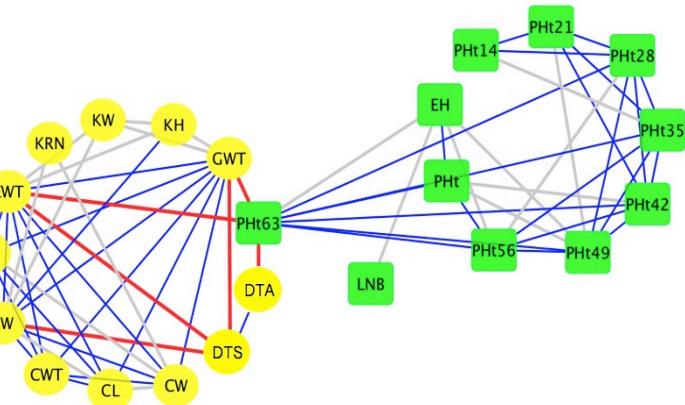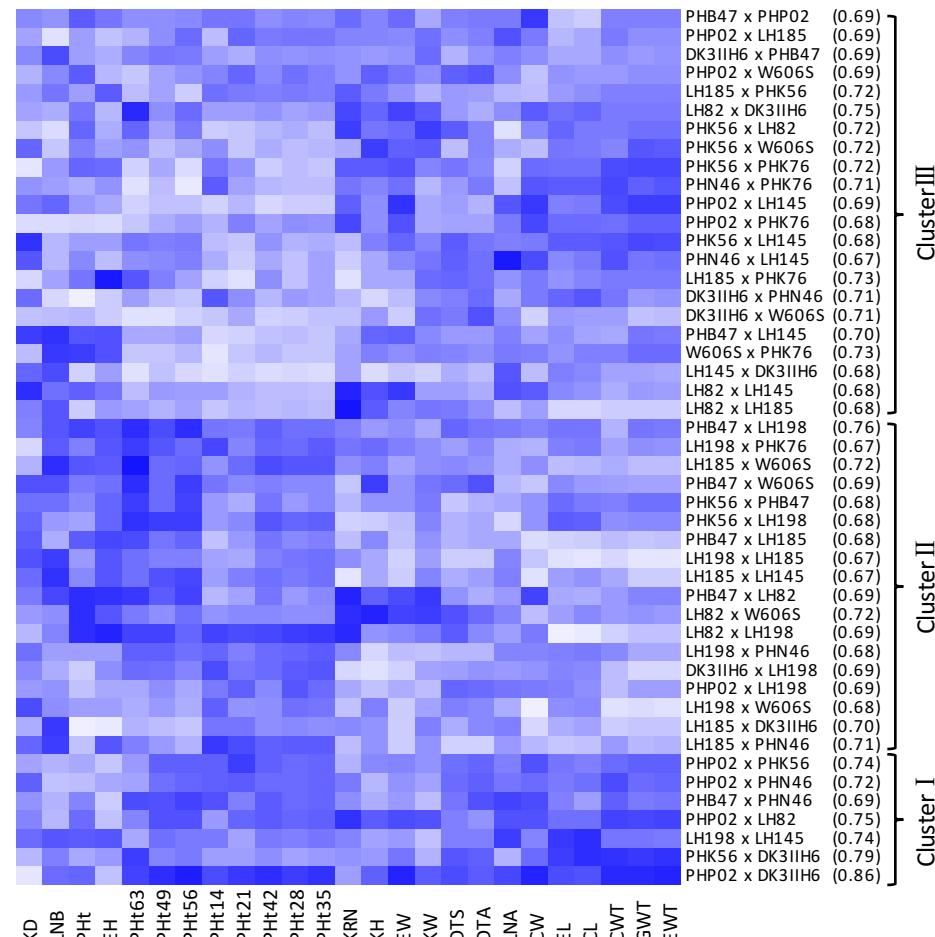
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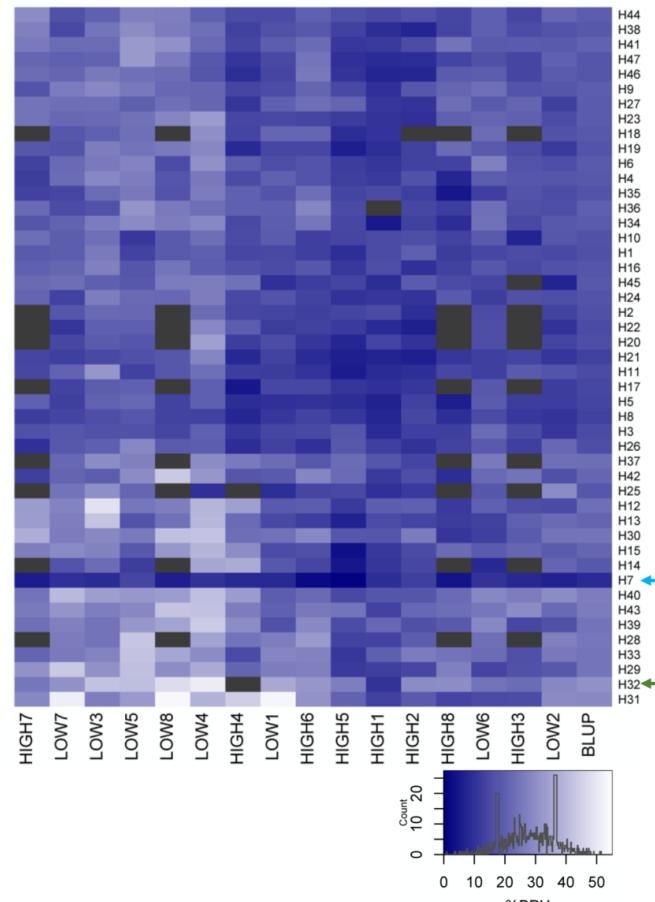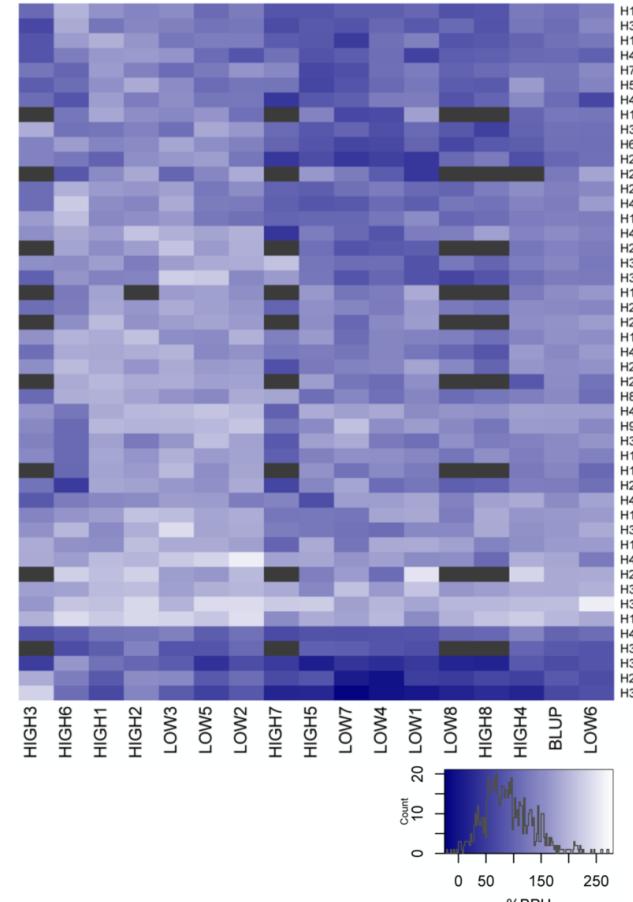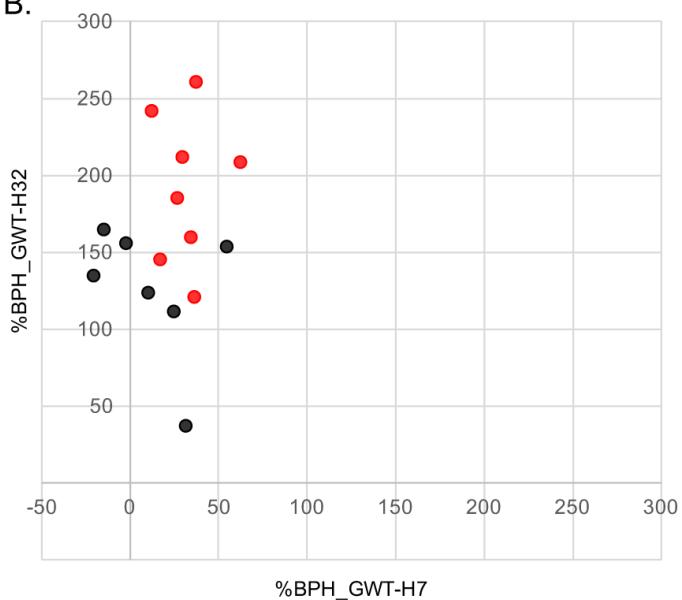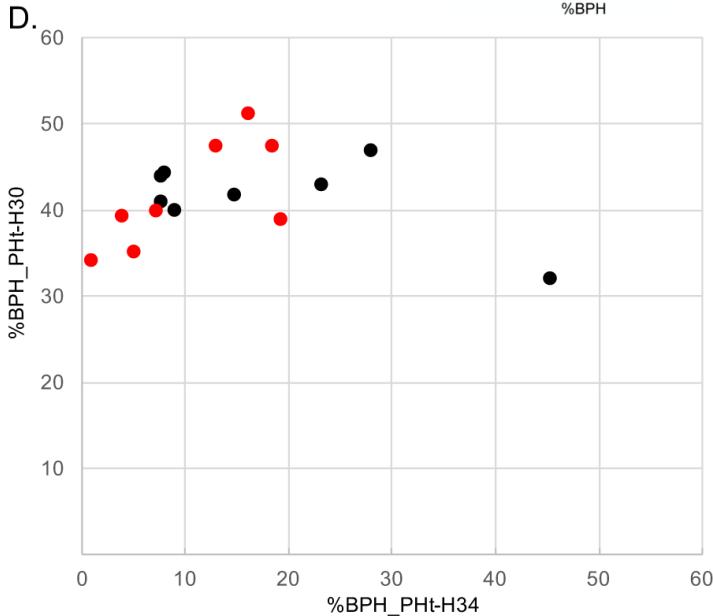
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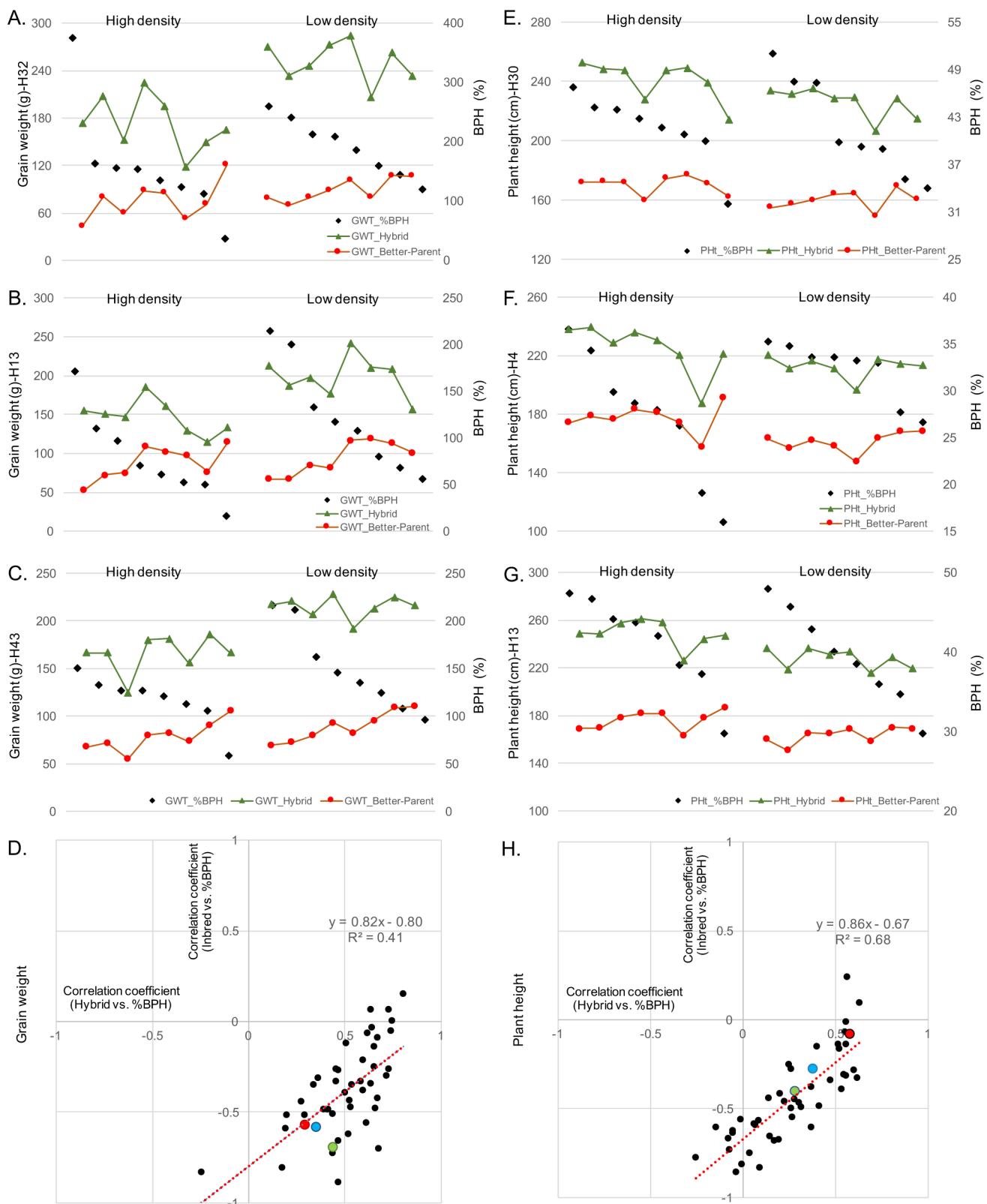
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