

Genetic and environmental determinants of phosphorus deficiency-induced heading delay in rice

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Abstract

A delay in heading under phosphorus (P) deficiency may affect the adaptation of rice to a range of environmental stresses by altering the reproductive development timing. However, how key heading time genes and their genetic networks are associated with phosphorus deficiency-induced heading delay (PDI-heading delay) remains unclear. Here, we examined 11 rice genotypes, including single- and double-mutant lines carrying functional and non-functional alleles of *Ghd7*, *Hd1*, *Ehd1*, and *Ef7*. Field trials were conducted under normal and low fertilizer conditions across multiple site-by-year environments (Env). PDI-heading delay was assessed as delta-days to heading (Δ DTH), calculated as the difference in days to heading between low-fertilizer (NF) and normal-fertilizer (NF) conditions. The mean Δ DTH across site-by-year environments was estimated at 7.76 d with a substantial variation among genetic backgrounds. In the single-gene effect on Δ DTH, only *Ef7* showed a significant effect whereby its non-functional allele delayed heading by 1.92 d than the functional allele. A two-gene interaction model identified *Ghd7* $\times$ *Hd1* as the most distinctly detected pairwise interaction. Significant *Hd1* $\times$ Env and *Ehd1* $\times$ Env interactions indicated that the effects of these genes varied between site-by-year environments. Our results suggest that PDI-heading delay is genetically distinct and is associated with specific heading time genes, pairwise gene interactions, and environmental context. These findings contribute to breeding efforts aimed at optimizing the phenological adaptation of rice plants to P deficiency.

Background

Phosphorus (P) deficiency is one of the most impactful soil constraints limiting lowland rice production in sub-Saharan Africa (SSA) (Saito et al. 2019b; Rakotoarisoa et al. 2020; Rakotoson et al. 2022). P deficiency suppresses root system development and reduces assimilate production, lowering biomass and yield (Dobermann and Fairhurst 2000). In addition to growth effects, P deficiency delays key phenological processes, including heading and maturity, underscoring its importance in crop production (Nord and Lynch 2008; Andrianary et al. 2021; IRRI, Rice Knowledge Bank).

The delay in phenological development associated with P deficiency has contrasting effects on crop production, depending on environmental conditions. In relatively stable and favorable growing environments, prolonged vegetative growth before heading can enhance biomass accumulation and, in some cases, increase grain yield (Nord et al. 2011; Andrianary et al. 2021). In contrast, when temperature decreases toward the end of the growing season in high-latitude and high-altitude regions, delayed heading induced by P deficiency may expose rice to low-temperature stress during the booting or flowering stages. This can lead to increased spikelet sterility and reduced yield (Rakotoarisoa et al. 2020; Andrianary et al. 2021). Therefore, P deficiency is a critical factor in mediating crop responses to climatic stress through its strong influence on phenological development.

However, previous agronomic studies have mainly focused on the effects of P deficiency on biomass, yield, and nutrient management (Buresh et al. 2010; Saito et al. 2019b). Meanwhile, the genotypic variation in heading time responses to P-deficient conditions in rice remains poorly understood. Such

variation is agronomically important because the timing of heading influences whether critical developmental stages coincide with biotic and abiotic stresses. Therefore, it may affect crop adaptation and yield stability (Nord et al. 2011; Andrianary et al. 2021; Rakotoson et al. 2022). In this context, identifying the genetic basis of this variation requires consideration of the heading time regulatory network through which rice responds to environmental signals.

Molecular genetic studies have established a complex photoperiodic heading time network in rice, including *Photosensitive 1* (*Se1*, also known as *Heading date 1* [*Hd1*]) (Chandraratna 1953; Yokoo and Kikuchi 1982; Yano et al. 2000), *E1* (also known as *Grain number, plant height and heading date 7* [*Ghd7*]) (Syakudo and Kawase 1953; Yamagata et al. 1986; Yano et al. 1997; Xue et al. 2008), *Early flowering 7* (*Ef7*, also known as *Heading date 17* [*Hd17*] or *OsELF3-1*) (Yuan et al. 2009; Saito et al. 2012; Matsubara et al. 2012; Zhao et al. 2012), and *Earliness 1* (*Ef1*, also known as *Early heading date 1* [*Ehd1*]) (Tsai 1976; Nishida et al. 2002; Doi et al. 2004). *Hd1* integrates daylength cues and can either promote or repress heading depending on the photoperiod. Meanwhile, *Ghd7* acts as a strong repressor under long-day conditions and influences vegetative growth and panicle traits (Xue et al. 2008; Hori et al. 2016). *Ehd1* links upstream regulatory cues to florigen output through *Hd3a* and *RFT1* (Doi et al. 2004; Komiya et al. 2008, 2009; Hori et al. 2016). *Ef7* functions in the photoperiod pathway and has been implicated in the regulation of *Ghd7* and heading time (Saito et al. 2012; Itoh et al. 2019). These factors form part of a regulatory network that translates environmental signals, particularly day length and temperature, into changes in the heading date. Their effects may depend on the allelic states of other genes and the growing environment, suggesting that environmentally responsive variations in heading time cannot be fully explained by independent single-gene effects. However, whether individual effects and pairwise gene interactions contribute to variations in heading delay under P-deficient conditions and whether these interactions vary across environments remain unclear.

In this study, we evaluated the heading time plasticity of rice genotypes carrying distinct functional and non-functional alleles of *Ghd7*, *Hd1*, *Ef7*, and *Ehd1*, including double mutants, under fertilized and low-fertilized conditions. Our objectives were to (i) quantify P-deficiency-induced heading delays (PDI-heading delays), defined as the difference in days to heading (DTH) between low-fertilized (LF) and normal fully fertilized (NF) conditions, and (ii) identify single-gene effects and pairwise genetic interactions among heading-time genes associated with this delay. These analyses provide insights into genotype-dependent phenological responses under P-deficient conditions and may support strategies for stabilizing rice production in environments where nutrient limitations and climatic stress coincide.

Results

Effects of single-genes and pairwise genetic interactions on DTH

The mean DTHs of WT, single mutant, and double mutant plants are summarized for the NF and LF treatments within each treatment in Table 1 and Supplemental Fig. S1.

All the genes, except *Hd1* showed significant single-gene effects on DTH (Table 2). The estimated marginal means indicated that the functional *Ghd7* allele was associated with late heading. Meanwhile, the functional alleles *Ehd1* and *Ef7* were associated with early heading (Fig. 1). The estimated DTH shortened from 76.7 to 51.9 d for *Ehd1* and from 70.1 to 58.4 d for *Ef7* when these alleles were functional. In contrast, the estimated DTH was prolonged by 3.0 d with the functional allele of *Ghd7*.

To determine whether DTH variation was also associated with combinations of heading time genes, we examined pairwise genetic interactions. In the pairwise genetic interaction model, the effects of *Ehd1* and *Ef7* were significant, whereas those of *Ghd7* and *Hd1* were not (Table 2). All the pairwise interactions between the four genes were significant, including *Ghd7* × *Hd1*, *Ghd7* × *Ehd1*, *Ghd7* × *Ef7*, *Hd1* × *Ehd1*, *Hd1* × *Ef7*, and *Ehd1* × *Ef7* (Table 2, Supplemental Fig. S2).

Single-gene effects on PDI-heading delay

Substantial variation was also observed in Δ DTH (Table 1, Supplemental Fig. S3). Across the tested materials, LF treatment significantly delayed heading, with the estimated mean Δ DTH being 7.76 d in the overall delay model (Fig. 2a). The estimated marginal means showed that Δ DTH varied among the tested genetic backgrounds. Relatively small delays were observed in HS110 and HEG2 with Δ DTH values of 5.16 and 5.64 d, respectively. Meanwhile, larger delays were observed in DMG8 and HS276 with Δ DTH values of 10.10 and 9.73 d, respectively (Fig. 2b).

Next, we examined whether the variation in Δ DTH could be explained by the additive effects of the four heading time genes. In the single-gene model, only *Ef7* showed a significant effect on Δ DTH after accounting for environmental differences. Meanwhile, the effects of *Ghd7*, *Hd1*, and *Ehd1* were not significant (Table 3).

Estimated marginal means indicated that the non-functional *ef7* allele was associated with a larger Δ DTH than the functional *Ef7* allele. The estimated Δ DTH was 9.04 d for *ef7* and 7.13 d for *Ef7*, corresponding to an approximately 1.92-d difference (Fig. 3). In contrast, the estimated Δ DTH values were similar between alleles of *Ghd7*, *Hd1*, and *Ehd1*. This is consistent with the absence of significant single-gene effects for these genes in the additive model.

Ghd7 × *Hd1* interaction was associated with PDI-heading delay

As the single-gene model explained only part of the genetic variation in Δ DTH, we next tested whether pairwise genetic interactions contributed to PDI-heading delay. In the pairwise interaction model, the main effects of *Ghd7* and *Ef7* and the pairwise interaction of *Ghd7* × *Hd1* were significant (Table 3).

Estimated marginal means for the *Ghd7* × *Hd1* combinations showed that Δ DTH was largest in the *ghd7* × *hd1* combination, with an estimated delay of 12.69 d (Supplemental Fig. S4). The estimated Δ DTH values were lower in the other combinations: 7.37 d for *Ghd7* × *hd1*, 8.40 d for *ghd7* × *Hd1*, and 8.14 d for

Ghd7 × *Hd1*. Pairwise comparisons indicated that the *ghd7* × *hd1* combination differed significantly from *Ghd7* × *hd1*. Meanwhile, other pairwise contrasts were not statistically significant after adjustment.

PDI-heading delay showed site-by-year-dependent genetic variation

To assess whether genetic effects on Δ DTH were environment-dependent, we analyzed gene-by-environment interactions using environment (Env) defined as each site-by-year combination. At the gene level, *Hd1* × Env and *Ehd1* × Env interactions were significant, whereas *Ghd7* × Env and *Ef7* × Env interactions were not significant in this model (Table 4). The main effect of *Ef7* remained significant, indicating that *Ef7* was associated with Δ DTH across environments. Meanwhile, the effects of *Hd1* and *Ehd1* on Δ DTH were more site-by-year dependent.

Exploratory site-specific gene-by-gene pairwise analyses further suggested that the pairwise interactions associated with Δ DTH differed among sites (Supplemental Table S1). The *Ghd7* × *Hd1* interaction was most evident at the February-sowing season in Ishigaki (I-Feb) site (Fig. 4), whereas the July-sowing season in Ishigaki (I-Jul) site exhibited a significant *Ghd7* × *Ef7* interaction (Supplemental Fig. S5). In Behenji, which is defined as cool-highland and low-P environment (Andrianary et al. 2021), *Ehd1*-related interactions were detected, although this result should be interpreted cautiously because only one year of data were available. These results indicate that the genetic control of the PDI-heading delay is modified by the site-by-year environment.

Discussion

Here, we showed that PDI-heading delay varied among genetic backgrounds and that site-by-year environments were associated with single-gene effects and pairwise genetic interactions among major heading time genes. The models detected different variables for DTH and Δ DTH, indicating relevant but distinct genetic mechanism to determine days to heading and the degree of delay under P deficiency among key heading time genes.

The environmental analyses further indicated that Δ DTH was modified by site-by-year environment. The results suggest that the effects of *Hd1* and *Ehd1* on Δ DTH varied among site-by-year environments, while *Ef7* showed a consistent main effect across environments. In the environmental summary analysis, mean temperature during the 45-d period after transplanting was negatively correlated with both DTH in NF treatment and Δ DTH. Meanwhile, day length showed no significant correlation with Δ DTH (Supplemental Fig. S6). This pattern suggests that temperature may contribute to site-by-year variations in LF-associated heading delays. *Ghd7*-mediated repression of heading is temperature-sensitive, with lower temperatures enhancing *Ghd7*-mediated repression and higher temperatures reducing this effect (Nagalla et al. 2021; Sun et al. 2022). Therefore, the temperature-dependent regulation of heading-time genes may partly explain why Δ DTH differs among environments. However, the 45-d period used in this study was selected as an exploratory window. Further analyses using additional environmental indices are necessary to identify the causal environmental drivers.

Among the pairwise genetic interactions, *Ghd7* × *Hd1* was the most distinctly detected pairwise interaction effect on Δ DTH. The significant *Ghd7* × *Hd1* interaction indicates that the contribution of *Ghd7* to Δ DTH depended, at least in part, on the allelic state of *Hd1*. A difference between *ghd7* and *Ghd7* was detected in the *hd1* background but not in the *Hd1* background. This pattern suggests that PDI-heading delay is influenced by the independent effects of individual heading time genes and by specific allele combinations.

Site-specific interaction patterns may reflect the positions of *Ghd7*, *Hd1*, *Ehd1*, and *Ef7* within photoperiodic heading pathways. *Ef7* (also known as OsELF3-1) functions as a component of the rice evening complex with OsELF4 and OsLUX (Xu et al. 2023). Previous studies have implicated this complex in the regulation of photoperiodic flowering pathways, including the regulation of *Ghd7* (Saito et al. 2012; Itoh et al. 2019; Xu et al. 2023). The detection of a *Ghd7* × *Ef7* interaction in I-Jul suggests that the contribution of *Ef7* to Δ DTH may depend on the *Ghd7* background under specific environmental conditions.

Hd1 can function as either a floral promoter or a repressor, depending on the genetic and environmental context. In addition, *Ghd7* and *Hd1* are involved in delaying heading under specific photoperiodic conditions (Nemoto et al. 2016; Zhang et al. 2017). These reports support the possibility that *Ghd7* and *Hd1* form an integration point where environmental and nutritional effects influence heading-time regulation. Similarly, the *Ghd7* × *Hd1* interaction detected in the pairwise interaction and site-specific analyses suggests that the combined state of these two heading time genes affects the PDI-heading delay.

A possible molecular link between nutrient status and heading-time regulation is provided by phosphate starvation signaling pathways. OsPHO2 interacts with the circadian regulator OsGI, suggesting a connection between P status and photoperiodic regulation (Li et al. 2017; Baek et al. 2026). In this context, LF treatments may indirectly influence heading time through phosphate signaling components that interact with the circadian or photoperiodic pathways. Based on the present phenotypic results and previously reported molecular relationships, we propose a working hypothesis in which phosphate starvation signaling interacts with photoperiodic and temperature-responsive pathways to modify heading time (Fig. 5). However, the present study did not directly examine gene expression, protein interactions, or phosphate signaling activity. Therefore, the proposed network should be regarded as a working hypothesis, requiring further molecular validation. Nevertheless, these findings provide a useful basis for identifying breeding targets to control heading time under P deficient condition in rice.

Conclusion

This study suggests that the PDI-heading delay is controlled by the combined effects of heading time genes, pairwise genetic interactions, and site-by-year environments. The *Ghd7* × *Hd1* interaction was the most distinctly detected pairwise interaction in the overall Δ DTH analysis and was especially evident in I-Feb. Meanwhile, other site-specific interactions were also observed. These results provide a framework

for understanding how nutrient-limited conditions interact with heading time gene networks to modify heading time in various environments. Although further validation is required, especially at the molecular level and under additional environmental conditions, allele combinations at heading time loci, such as *Ghd7*, *Hd1*, *Ehd1*, and *Ef7*, may be useful candidates for future studies aimed at adjusting the heading time under low-fertility or P deficient conditions.

Materials and Methods

Plant materials

We used 11 genotypes, including a *japonica* variety ‘Gimbozu’ as original wild type (WT), four single heading time mutants or derivative lines, and six double-mutant lines (DMGs) (Table 5). HEG2 is a near-isogenic lines at the *E1* (*Ghd7*) locus in the ‘Gimbozu’ background, harboring the early allele *e1* (*ghd7*) introgressed from ‘Aikoku’ (the *e1* donor) (Yamagata et al. 1986; Saito et al. 2019a). HS110 is an early-heading mutant of ‘Gimbozu’ carrying a loss-of-function allele *se1* (*hd1*) at the *Se1* (*Hd1*) locus (Tanisaka et al. 1992; Yano et al. 2000). The late-heading mutant HS169 harbors the non-functional allele *ef1-h* (*ehd1*) at the *Ef1* (*Ehd1*) locus (Nishida et al. 2002; Doi et al. 2004; Saito et al. 2009). Meanwhile, HS276 carries the non-functional allele *ef7* at the *Ef7* (*Hd17*) locus (Yuan et al. 2009; Saito et al. 2012; Matsubara et al. 2012).

We designed allele-specific DNA markers for all four loci and confirmed their genotypes using marker-assisted selection. By intercrossing four single-mutant lines and selecting progeny fixed for both target loci, we developed six DMGs. All the DMGs advanced to homozygosity through self-pollination combined with marker-based selection.

Genotyping was performed using locus-specific PCR, allele-specific PCR, or PCR–RFLP assays (Supplementary Table S2). Expected band sizes for the wild-type and mutant genotypes are shown. For *Ef7*, *AfaI* digestion of the 207-bp amplicon yields a 191-bp fragment specific to the mutant allele, whereas the wild-type allele remained undigested. For *Ghd7*, a three-primer allele-specific PCR assay discriminated between the wild-type (350 bp) and mutant (450 bp) alleles in a single reaction. For *Hd1* and *Ehd1*, PCR assays differentiated the presence or absence of the *mPing* insertion (433bp) based on the amplicon size.

Experimental design

Ten lines (four single mutants, six double mutants) and the recurrent parent ‘Gimbozu (= WT)’ were grown under irrigated lowland conditions in two cropping seasons, that is, I-Feb and I-Jul. Sowing was conducted in February and July, respectively, at an experimental paddy field at JIRCAS, Tropical Agriculture Research Front in Ishigaki, Okinawa, Japan (24.38°N, 124.19°E). For another single cropping season at farmers’ paddy fields in a commune of Behenjy, located in the central highlands of Madagascar (19.10°S, 47.29°E). Behenjy was included as a contrasting highland environment to evaluate whether heading time responses to P-deficient conditions (Andrianary et al. 2021), and their effects

differed from those observed under the subtropical conditions of Ishigaki. Hereafter, each combination of site/cropping season and year is referred to as an environment (Env), resulting in seven Env events: I-Feb. 2021, I-Feb. 2022, I-Feb. 2023, I-Jul 2020, I-Jul 2022, I-Jul 2023, and Behenjy 2024. Each cropping schedule is summarized in Supplemental Table S3. The daily mean temperature and day length after transplantation are shown in Supplemental Fig. S7.

Normally fully fertilized (NF) and low-fertilized (LF; mainly focused on P deficiency) fields were established in each environment. In Ishigaki, the NF and LF plots were established on low-fertility soil prepared by incorporating subsoil from beneath the plow layer. Fertilizer was applied only to the NF plot. Soil chemical analyses conducted before transplanting indicated that the available P was substantially lower in the LF plot than in the NF plot. Meanwhile, the other major nutrients were less affected (Supplemental Table S3, Ueda et al. 2024). The NF plot received compound chemical fertilizers (5.2-5.2-5.2 g/m² N-P-K) as a basal application. The LF plot did not receive fertilizer throughout the growing season. In Behenjy, the amount of available P was the lowest among the lowland and upland rice fields in Madagascar (Andrianary et al. 2021). The NF plots received nitrogen fertilizer (6 g/m²) as urea and phosphorus fertilizer (3 g/m²) as triple superphosphate as a basal application, with an additional nitrogen fertilizer (3 g/m²) at 60 days after transplanting. In contrast, the LF plots received only nitrogen fertilizer (6 + 3 g/m²).

In Ishigaki, split-plot designs in two cropping seasons were applied with two levels of fertilizer treatment in the main plot and 11 genotypes in the sub-plots. The experiments were conducted over three years. Within each year, one to three blocks (replicates) were established per fertilizer treatment (NF and LF). Therefore, the year was nested as a random effect. The size of sub-plot was 0.3 m × 1.8 m. Approximately one-month old seedling was transplanted with one seedling per hill and at the density of 18.5 hills per/m² (30 cm × 18 cm). In Behenjy, a split-plot design was applied to three replicates, with two fertilizer treatments (NF and LF) in the main plot and 11 genotypes in the sub-plot. The size of subplot was 1.5 m × 1.8 m. Approximately one-month old seedling was transplanted with one seedling per hill and at the density of 33.3 hills/m² (15 cm × 20 cm).

Phenology measurement

Heading was defined at the plant level as the emergence of the first panicle from the flag leaf sheath. For each plot, the days to heading (DTH) were calculated as the number of days from transplantation until 50% of the plants had headed. The plot-level mean DTH was used as the experimental unit for all statistical analyses. Δ DTH was calculated as DTH under LF minus DTH under NF for each genotype within the same Env and block whenever paired observations were available. Δ DTH is hereafter referred to as PHD-heading delay.

Statistical analysis

All statistical analyses were conducted using the R software (version 4.3.2). Linear mixed-effects models were fitted using the *lme4* package (Bates et al. 2015), and the significance of fixed effects was

assessed using Type III analysis of variance (ANOVA) with Satterthwaite's approximation for degrees of freedom, as implemented in the *lmerTest* package (Kuznetsova et al. 2017). The sum-to-zero contrast was used as a categorical predictor. The estimated marginal means and pairwise comparisons were performed using the *emmeans* package (Lenth 2016). Tukey's adjustment was used for multiple comparisons among the estimated marginal means. The model assumptions were evaluated by visually inspecting the residuals and fitted values.

For the DTH, Env was defined as a site-by-year combination. Fertilizer conditions, Env, and genotype or gene effects were treated as fixed effects. The block nested within Env was included as a random effect, when applicable. To evaluate the baseline genetic architecture of DTH, two models were fitted. The single-gene model included the additive effects of *Ghd7*, *Hd1*, *Ehd1*, and *Ef7* together with fertilizer conditions and Env. The pairwise genetic interaction model included the fertilizer condition, Env, the additive effects of the four genes, and all pairwise interactions among the four genes. A genotype model, including genotype as a fixed effect, was also fitted as a supporting analysis.

To evaluate PHI-heading delay, Δ DTH was analyzed as the primary response variable. Genotype, single-gene, pairwise genetic interaction, and gene-by-environment models were fitted separately because they address distinct biological questions. This hierarchical approach was used to distinguish variations among genetic backgrounds from additive gene effects, pairwise genetic interactions, and environment-dependent gene effects, while retaining model interpretability and avoiding overparameterization.

First, genotype and Env effects on Δ DTH were tested using a model including genotype and Env as fixed effects and block nested within Env as a random effect. Next, single-gene and pairwise genetic interaction models were fitted for Δ DTH. The single-gene model included Env and the additive effects of *Ghd7*, *Hd1*, *Ehd1*, and *Ef7*. The pairwise interaction model included Env, the additive effects of the four genes, and all pairwise interactions among the four genes.

To test whether genetic effects on Δ DTH depended on environment, a gene-by-environment model was fitted. This model included Env, the additive effects of *Ghd7*, *Hd1*, *Ehd1*, and *Ef7*, and the interactions between each gene and Env. Gene-by-environment interactions were evaluated to determine whether environmental responses differed among the tested genetic backgrounds.

Site-specific $G \times G$ models were subsequently used as exploratory analyses to assess possible variations in pairwise genetic interactions among sites. Models for the site-specific exploratory analyses were fitted separately for I-Feb, I-Jul, and Behenjy. For I-Feb and I-Jul, the year was included as a random effect. For Behenjy, which had one year of data, a linear model without year random effects was fitted. Site-specific models were used to evaluate additive gene effects and pairwise genetic interactions. Site-specific models were fitted to selected pairwise interactions to visualize the estimated marginal means.

Environmental relationships were evaluated using Pearson's correlation coefficients between environment-level mean DTH or Δ DTH and environmental variables. Daily mean temperature and day length are summarized for the 45-d period after transplantation. Each point in the correlation analysis

represents the mean trait value across genotypes in each site-year environment. For all analyses, $P < 0.05$ was considered statistically significant unless otherwise stated.

Abbreviations

ANOVA	
Analysis of Variance	
DMG	
Double-mutant line	
DTH	
days to heading	
Env	
Environment	
$G \times E$	
gene-by-environment interaction	
$G \times G$	
gene-by-gene interaction	
I	
Feb-February-sowing season in Ishigaki	
I	
Jul-July-sowing season in Ishigaki	
LF	
lower fertilizer conditions	
NF	
Normal-fertilizer conditions	
PDI	
Phosphorus deficiency-induced	
WT	
wild-type	
ΔDTH	
difference in days to heading between LF and NF conditions (DTH under LF minus DTH under NF)	

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors’ contributions

HS and YT conceptualized and designed the research; YU, and TI provided guidance on experimental methodology. HS, BHA and YT collected data performed the experiments; HS and BHA analyzed the data and created the figures and tables; YU, and TI contributed to data interpretation; HS wrote the initial manuscript draft. All authors read, revised, and approved the manuscript.

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Tables

Tables 1 to 4 are available in the Supplementary Files section.

Figures

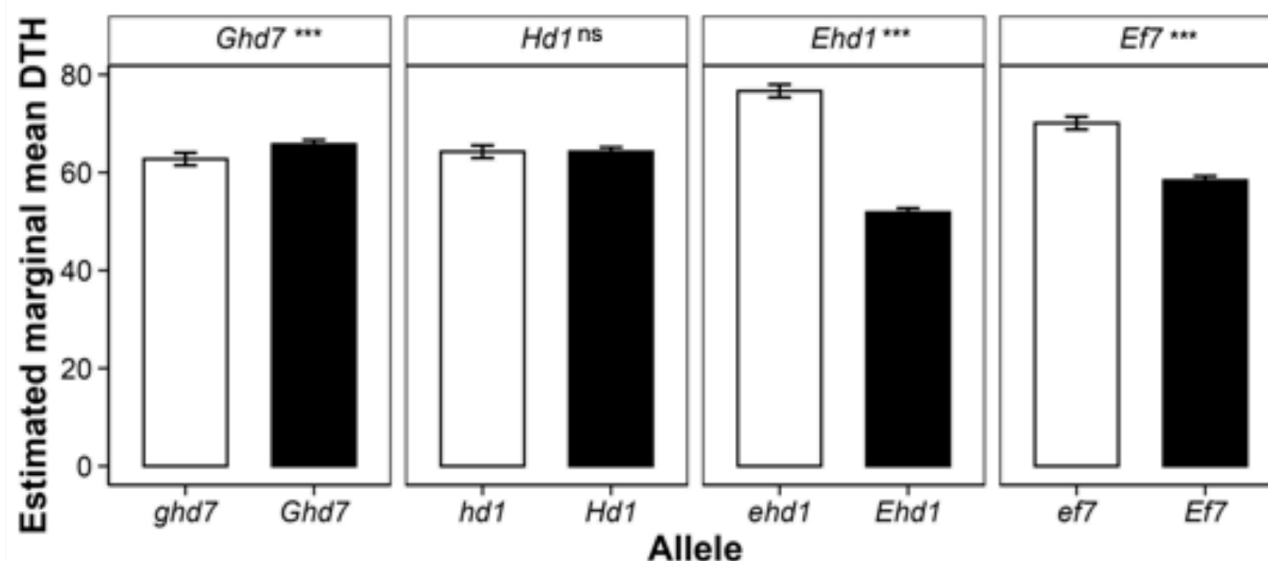


Fig. 1

Figure 1

Effects of single heading-time genes on DTH.

The estimated marginal mean DTH for the functional and non-functional alleles of *Ghd7*, *Hd1*, *Ehd1*, and *Ef7*. Bars represent the estimated marginal means from the single-gene model and error bars indicate 95% confidence intervals. White bars indicate non-functional alleles and black bars indicate functional alleles. Asterisks to the right of the genes indicate significance levels for the allele effect in the single-gene model in Table 3: *** $P < 0.001$; ns, not significant. The model included fertilizer conditions and Env as adjustment factors. Env is defined as the site-by-year combination.

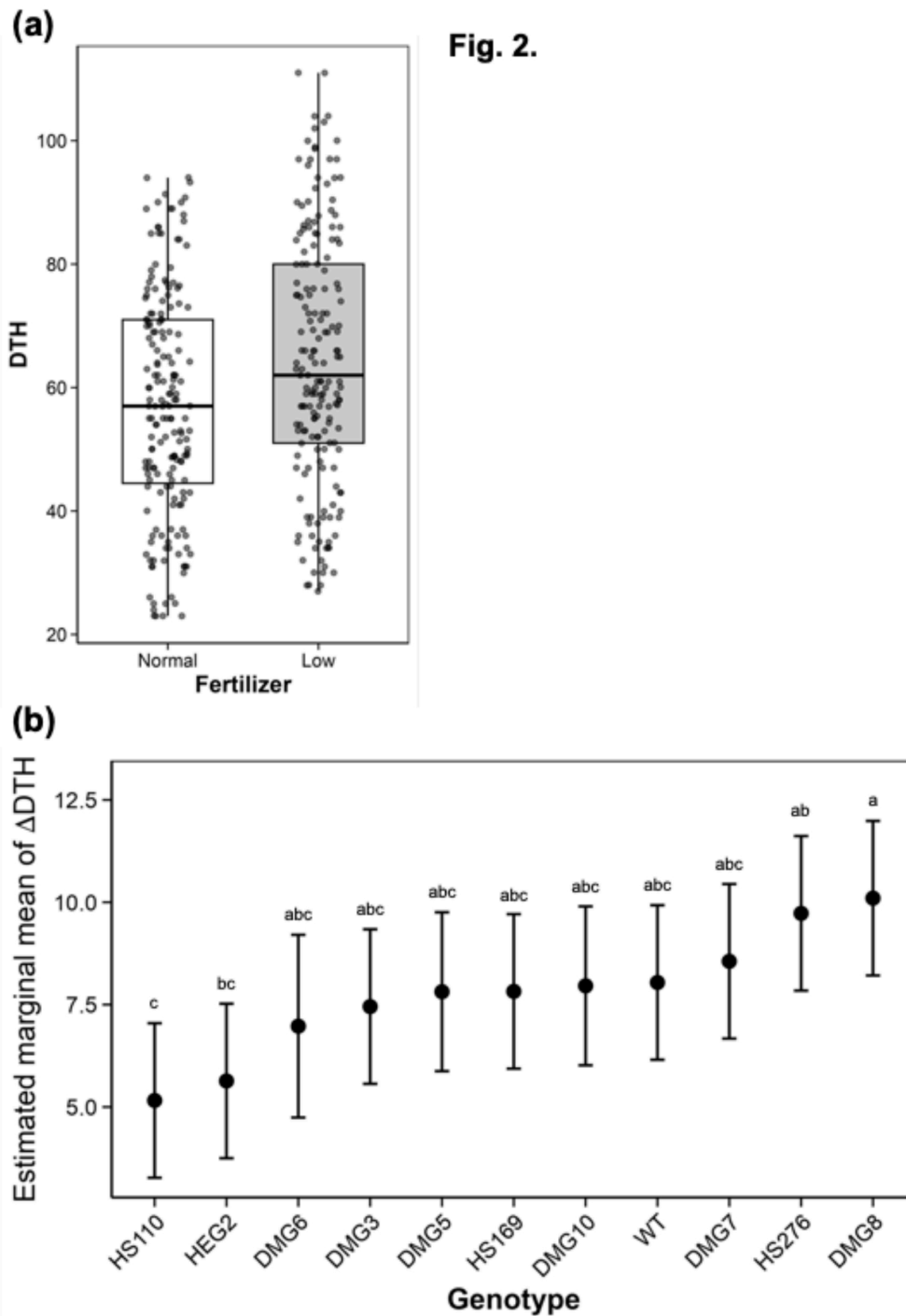


Figure 2

PDI-heading delay varies among genetic backgrounds.

(a) Observed DTH under NF and LF conditions. Boxplots show the distribution of observed DTH values, and dots indicate individual observations. White and gray boxes indicate normal fertilization (NF) and low-fertilization (LF) conditions, respectively.

(b) Estimated marginal mean Δ DTH for each genotype. Points represent estimated marginal means from the genotype model, and error bars indicate 95% confidence intervals. Different letters indicate significant differences among genotype estimated marginal means based on Tukey-adjusted comparisons.

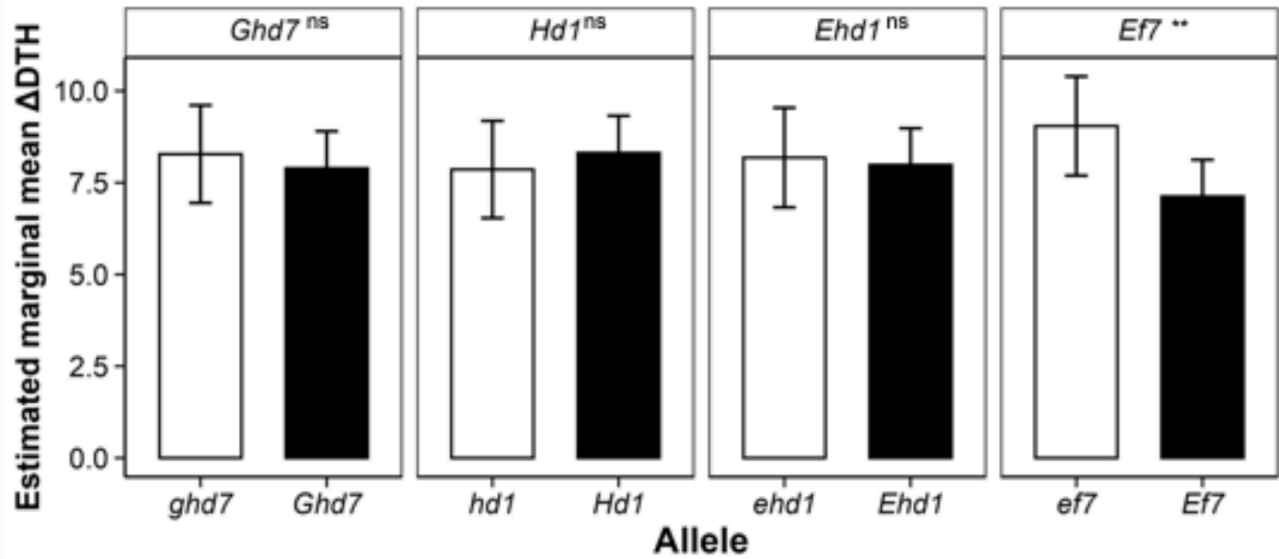


Fig. 3.

Figure 3

Single-gene effects on Δ DTH.

Estimated marginal mean Δ DTH for functional and non-functional alleles of *Ghd7*, *Hd1*, *Ehd1*, and *Ef7*. Bars represent estimated marginal means from the single-gene Δ DTH model, and error bars indicate 95% confidence intervals. White bars indicate non-functional alleles and black bars indicate functional alleles. Asterisks to the right of the genes indicate significance levels for the allele effect in the single-gene model in Table 4: ** $P < 0.01$; ns, not significant. The model included Env as an adjustment factor. Env is defined as the site-by-year combination.

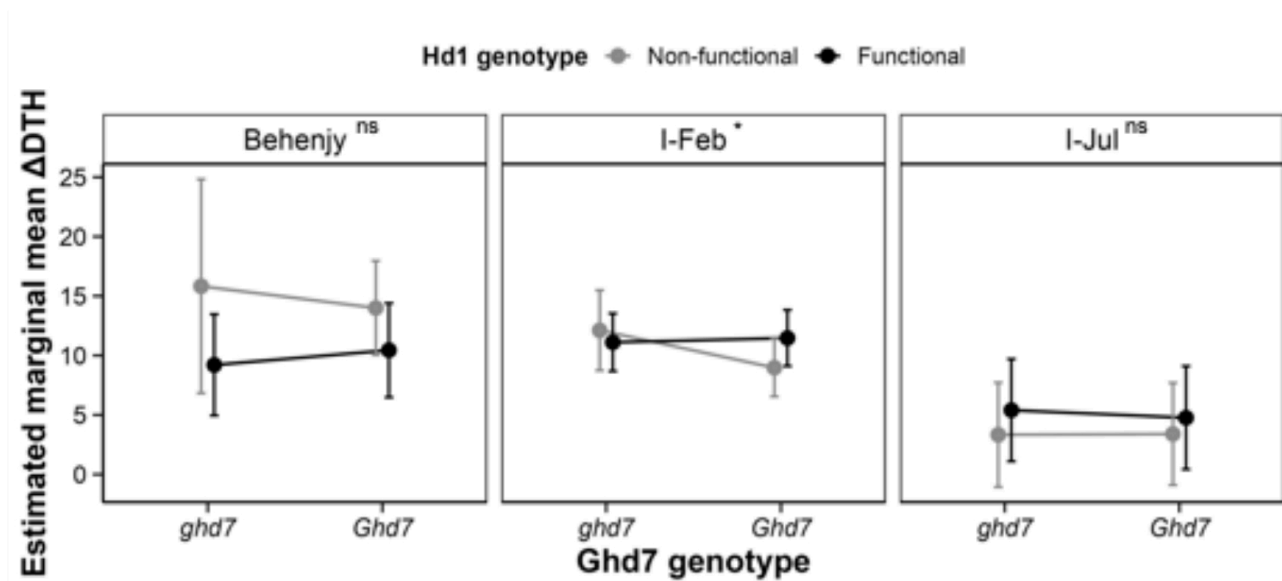


Fig. 4.

Figure 4

Site-specific *Ghd7* × *Hd1* interaction on ΔDTH .

Estimated marginal mean ΔDTH is shown for each *Ghd7* genotype in the *Hd1* and *hd1* backgrounds at each site. Points represent estimated marginal means and error bars indicate 95% confidence intervals. Gray and black lines indicate non-functional *hd1* and functional *Hd1* backgrounds, respectively. Estimates were obtained from site-specific focused models including *Ghd7*, *Hd1*, and their interactions. The year was fitted as a random effect for Feb and I-Jul. For Behenji, which had only one year of data, a linear model without a year-long random effect was used. Asterisks to the right of the sites indicate the significance of the *Ghd7* × *Hd1* interaction term in each site-specific model (* $P < 0.05$; ns, not significant).

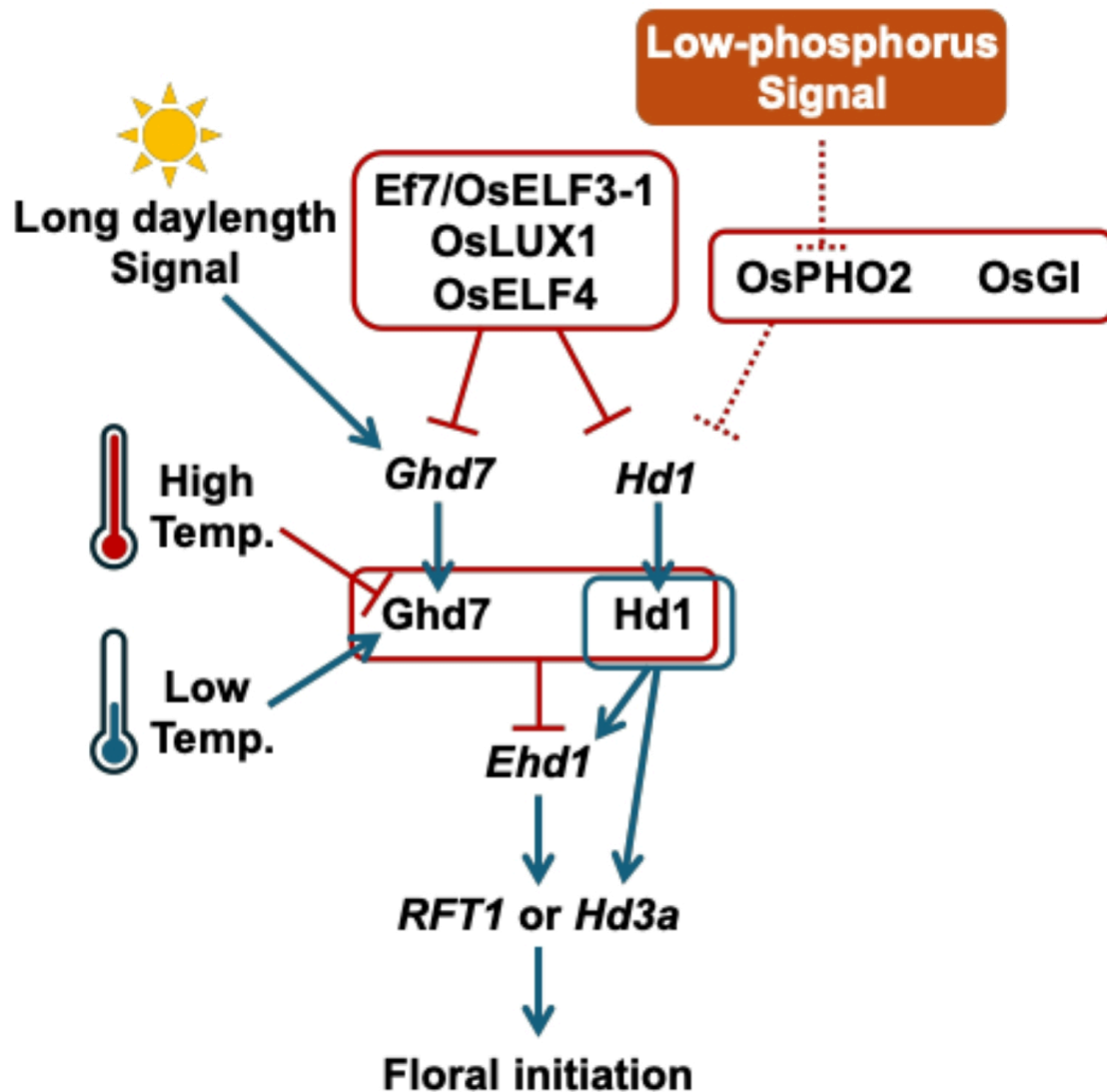


Fig. 5.

Figure 5

Working hypothesis for environment- and genotype-dependent regulation of heading time under phosphorus deficient conditions.

The schematic integrates the phenotypic results of the present study with previously reported relationships between phosphate homeostasis, the circadian clock, temperature, and photoperiodic pathways. Red bars indicate the repression of downstream gene expression or function, whereas blue arrows indicate activation. Red-outlined boxes represent protein complexes with repressive functions, and blue-outlined boxes represent complexes or factors with promotive functions. Solid arrows denote

the regulatory interactions supported by genetic or experimental evidence, whereas the overall network summarizes the working hypothesis derived from the present study. The red dashed T-bars indicate putative links that were not directly tested in this study.

Supplementary Files

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