$Hd3a$ and $RFT1$ integrate photoperiodic and drought stress signals to delay the floral transition in rice

Running title, Integration of drought and photoperiod in rice

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ABSTRACT

Plants show a high degree of developmental plasticity in response to external cues, including day length and environmental stress. Water scarcity in particular can interfere with photoperiodic flowering, resulting in the acceleration of the switch to reproductive growth in several species, a process called drought escape. However, other strategies are possible and drought stress can also delay flowering, albeit the underlying mechanisms have never been addressed at the molecular level. We investigated these interactions in rice, a short day species in which drought stress delays flowering. A protocol that allows the synchronization of drought with the floral transition was set up to profile the transcriptome of leaves subjected to stress under distinct photoperiods. We identified clusters of genes that responded to drought differently depending on day length. Exposure to drought stress under floral-inductive photoperiods strongly reduced transcription of *EARLY HEADING DATE 1* (*Ehd1*), *HEADING DATE 3a* (*Hd3a*) and *RICE FLOWERING LOCUS T 1* (*RFT1*), primary integrators of day length signals, providing a molecular connection between stress and the photoperiodic pathway. However, phenotypic and transcriptional analyses suggested that *OsGIGANTEA* (*OsGI*) does not integrate drought and photoperiodic signals as in Arabidopsis, highlighting molecular differences between between long and short day model species.

Keywords: rice, photoperiodic flowering, drought stress, florigen, *Ehd1*, *OsGI*, RNA-Sequencing
INTRODUCTION

Water is a precious and limited resource. Of all water available, only 1% is fresh water and is mostly used in agricultural practices. Finding sustainable solutions to water usage will be a priority in the near future to sustain food supplies of a growing population. Rice provides staple food for half of the world population. Its cultivation has been mostly optimized under semi-aquatic conditions in paddy fields, where the demand for water is extremely high and the production of a Kg of seeds can require up to 5000L of water (Todaka et al. 2015). However, more than 30% of rice is cultivated under rainfed areas that are subject to frequent water shortages (Dixit et al. 2014). Drought can be considered as one of the most prominent abiotic stresses in agriculture, affecting different aspects of plant development and productivity (Yamaguchi-Shinozaki & Shinozaki, 2006). Understanding how rice responds to drought and how water deprivation can affect key developmental processes is of fundamental importance to design more tolerant and resilient varieties.

Plant responses to dehydration cause changes at the physiological, morphological and molecular level, including altered transcription patterns of many genes (Shinozaki & Yamaguchi-Shinozaki, 2007, Rabbani et al. 2003; Ray et al. 2011; Maruyama et al. 2012). Drought responsive genes can be divided into two groups based on their involvement in protecting cells against environmental stress or in regulating genes that transduce stress response signals (Ingram et al. 1996; Shinozaki & Yamaguchi-Shinozaki, 2000; Minh-Thu et al. 2013). The first group includes water channel proteins, lipid desaturases and enzymes catalyzing the biosynthesis of osmoprotectants such as glycerol, mannitol, sucrose and proline (Ray et al. 2011; Minh-Thu et al. 2013). The second comprises master regulatory proteins including transcription factors, protein kinases and chromatin remodeling factors (Agarwal et al. 2006; Nakashima et al. 2009; Yu et al. 2013; Han & Wagner 2014).
Increasing evidences document that drought also impacts on the flowering process in diverse species (Sherrard & Maherali, 2006; Bocco et al. 2012; Franks, 2011; Bernal et al. 2011; Ivey & Carr, 2012; Kobayashi et al. 2013; Riboni et al. 2013). Several plants, including Arabidopsis thaliana, wheat and barley adopt a drought escape strategy whereby water deprivation rapidly induces flowering and seed set, in order to complete the life cycle before stress conditions become lethal (Mc Master & Wilhelm, 2003; Sherrard & Maherali, 2006; Franks et al. 2007; Bernal et al. 2011; Franks, 2011). Conversely, other species respond to drought by delaying flowering, eventually resuming it as environmental stress is over. Therefore, regulatory connections exist between the drought response and floral induction pathways. It is however unclear if they are shared between species that deploy different strategies to cope with drought stress.

Flowering of Arabidopsis is rapidly induced when plants are exposed to long days (LD), and a genetic cascade comprising the GIGANTEA (GI), FLAVIN BINDING KELCH REPEAT F-BOX PROTEIN 1 (FKF1), CONSTANS (CO) and CYCLING DOF FACTOR genes (CDFs) activates the transcription of FLOWERING LOCUS T (FT) and TWIN SISTER OF FT (TSF), components of the florigenic signal (Andrés & Coupland, 2012). Drought applied under LD triggers a drought escape response that is not observed under short days (SD) or in plants where GI or FT and TSF are mutated (Riboni et al. 2013).

Flowering in rice (also called heading) is activated under short photoperiods, whereas long days have a repressive effect on the floral transition. This type of photoperiodic response, despite being opposite from that of Arabidopsis, depends on a genetic cascade that shares the same components (Shrestha et al. 2014). Under SD, the OsGI protein induces expression of HEADING DATE 1 (Hd1), a homolog of CO, that in turn activates the transcription of HEADING DATE 3A (Hd3a) and RICE FLOWERING LOCUS T 1 (RFT1), homologs of FT (Hayama et al. 2003; Komiya et al. 2008). Rice evolved a parallel inductive pathway that can
promote expression of $Hd3a$ and $RFT1$, and is dependent on the function of *EARLY HEADING DATE 1* (*Ehd1*) (Doi et al. 2004). Under non-inductive LD, *Hd1* represses the transcription of *Ehd1*, *Hd3a* and *RFT1*, delaying the floral transition (Gómez-Ariza et al. 2015). The dual role of *Hd1* as SD activator and LD repressor of the flowering process is a feature not shared by Arabidopsis *CO*.

Heading of rice plants is delayed upon exposure to drought. The effect has been reported in different varieties and upon stressing at different developmental stages (Fisher & Fukai 2003; Ji et al. 2005; Bocco et al. 2012). Despite the key importance of correct heading dates for reproductive success, it is currently unclear (i) how rice plants respond to water deprivation during photoperiodic induction and (ii) whether day length affects the response to abiotic stresses. Finally, it is not clear if components of the photoperiodic network are involved in the flowering response of rice plants under drought stress, and whether the conclusions obtained using Arabidopsis as model system constitute a widely applicable frame for studying the interaction between drought and the flowering process in monocot species adapted to SD.

In this study, we explored the effects of drought stress applied to rice plants grown under specific photoperiods and during floral commitment, when plants switch from vegetative to reproductive growth. Transcriptional responses were assessed at the genome-wide scale and genes differentially responding to drought depending on the photoperiod were identified. *Ehd1*, *Hd3a* and *RFT1* were identified as points of convergence of flowering and drought signals in rice. However, the drought response was not altered in *osgi* mutants, underlying distinct responses between rice and Arabidopsis.

**MATERIALS AND METHODS**

**Plant material and growth conditions**
Nipponbare (NB) seeds were used for all the experiments. Plants were grown in Conviron PGR15 chambers set on temperature/relative humidity cycles of 28ºC/80% during the day and 24ºC/90% during the night. Light was provided by fluorescent tubes and metal halide bulbs (intensity of ~450μE/m²·sec⁻¹). The mutant line AB156681 corresponding to the OsGI locus and named osgi-3 was obtained from the National Institute of Agrobiological Sciences of Japan (https://tos.nias.affrc.go.jp/). Mutant plants were genotyped using primers specific for tos17 5’-GTACTGTATAGTTGGCCCATGTCC-3’ and OsGI 5’-CCTGCGTTCTGCTCACATACCT-3’. The hd1-1 and hd1-2 mutant alleles were previously described (Gómez-Ariza et al. 2015). Heading date measurements were obtained from at least 15 plants per genotype.

Drought stress assays

One hundred seeds of NB were planted on soil in 10L square boxes and plants were grown for 4 weeks under LD conditions and a normal water regime. After 4 weeks, half of the pots were moved to SD. Under each photoperiod, half of the pots were watered normally (control), while the remaining half was subjected to drought stress. Soil water content was monitored every hour using moisture sensors (WaterScout SM 100 Soil Moisture Sensor® and WatchDog A-Series Loggers®). The relative water content (RWC) was measured according to (Baldoni et al. 2013). The analysis of covariance (ANCOVA) was applied to RWC data using plant height as covariate and α=0.05. Finally, expression of drought responsive genes DEHYDRATION-RESPONSIVE ELEMENT BINDING PROTEIN 2A (OsDREB2A), Dehydrin 1 (OsDhn1) and OsNAC6 was quantified in each experiment using a Mastercycler Realplex² (Eppendorf).
For LD and SD diurnal time course experiments, seeds were planted in two distinct 10L square pots and watered normally or exposed to drought stress. Samples were collected after 8 or 6 weeks from plants grown under LD and SD, respectively.

**RNA extraction and quantification of mRNA expression**

The distal part of the last extended leaf of 2-3 plants was collected and total RNA was isolated and quantified according to (Gómez-Ariza et al. 2015). One µg of total RNA was retro-transcribed using ImProm-II™ Reverse Transcriptase (Promega) with oligo-dT. Synthesized cDNAs were used as templates to quantify gene expression using the 2X Maxima SYBR Green qPCR Master Mix (Thermo Scientific) in a Mastercycler® ep Realplex² (Eppendorf). Quantification of cDNA was standardized using *Ubiquitin* and calculated using the $2^{-\Delta\Delta Ct}$ method. Primers used to quantify gene expression are listed in Supporting Information Table 2.

**RNA sequencing and data processing**

Total RNA was extracted using the NucleoSpin® RNA Plant (Macherey-Nagel) kit and total RNA was treated and quantified as above. Sequencing was performed at BGI Tech Solutions (Hong Kong) using an Illumina HiSeq 2000. RNA-seq yielded 24 to 37 millions of cleaned 50 bp single reads (Supporting Information Table S1). Quality of raw data was checked using the FastQC tool for high throughput sequence data (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). About 98% of the cleaned reads were aligned against the *O. sativa* Japonica group cultivar Nipponbare genome version 7.0 (http://rice.plantbiology.msu.edu/) using bowtie2 and TopHat2 (Langmead & Salzberg 2012, Kim et al. 2013). About 90% of the reads were uniquely mapped and counted by HTSeq (Anders et al. 2015) and subsequently used for gene-level differential expression analysis.
using the R software (version 3.1.2) for statistical computing and edgeR package version 3.6.8 from bioconductor (Robinson et al. 2010, Gentleman et al. 2004). For subsequent analyses, only features with more than 1 read per million in at least 3 samples were retained, for a total of 23,672 genes expressed across all conditions. Differentially expressed (DE) genes were called using a 1% false discovery rate (FDR) control and filtered for log2 fold change (FC) values below or above 1.5 yielding 8,359 DE genes. Gene Ontology enrichments were performed using PANTHER (Mi H. et al. 2013) from the GOC website (http://geneontology.org). Circular data visualization was obtained with the circus software package (Krzywinski et al. 2009).

RESULTS

Synchronizing the floral transition and drought stress

A system was set up to synchronize drought with the early stages of floral induction in the rice reference variety Nipponbare (NB). About 100 plants/treatment were grown for 4 weeks under LD conditions and two groups were then shifted to SD in order to induce flowering. Under these conditions, about thirteen days are sufficient for floral commitment in NB (F.G. and F.F. unpublished data). Drought stress was applied to plants grown under LD or shifted to SD by progressively reducing water content in the soil. Soil sensors measuring the volumetric water content (VWC%) were used to design water reduction curves, whose slope was monitored daily and adjusted to reach zero after thirteen days (Fig. 1a, 1b). The relative water content (RWC) of leaves was not affected by the reduction in VWC% 6 days after the beginning of the treatment, showing values above 80% (Fig. 1a, 1b). However, after thirteen days, plants showed a statistically significant decrease in the RWC and displayed completely rolled leaves (Fig. 1c-1e). Plants were recovered from drought stress and by day 19 the RWC
values had reached pre-drought levels (Fig. 1a, 1b). All plants survived and were able to complete their life cycle.

The molecular response to drought was monitored by quantifying the mRNA expression levels of drought-responsive genes, including OsDREB2A, OsNAC6 and OsDhn1 (Dubouzet et al. 2003; Nakashima et al. 2007; Lee et al. 2005). All genes showed a marked increase of expression after 13 days of drought compared to control plants (Fig. 1f-1h). Response to the SD treatment was monitored by quantifying Hd3a expression that was increased 13 days after the shift to SD (Fig. 1i). Finally, heading dates were scored and a statistically significant and reproducible delay was observed in plants exposed to drought stress under SD conditions (Fig. 1j). After recovery, plants set seeds normally and fertility was similar between treated and untreated plants (data not shown). In conclusion, this protocol provides a reliable method to assess the interaction between drought stress and photoperiodic induction in soil-grown plants.

Transcriptional profiling of leaves exposed to long or short days under drought stress

The global transcriptional effects of drought stress were monitored in leaves using RNA-sequencing. RNA was extracted from leaves subjected to dual treatments according to the scheme of Fig. 2a. Differentially expressed genes were uniformly distributed along the chromosomes and most of them were induced or repressed independently of the photoperiod with 5,937 genes (71%) in common between samples subjected to drought stress under SD and LD treatments (Fig. 2b, 2c). The number of genes differentially expressed in response to drought was overall greater (7,854) than the number of genes controlled by day length (1,645), suggesting a major impact of water deficit on global transcription in the leaves (Supporting Information Table S1). The proportion of genes downregulated by drought was always higher (59% under LD and 57% under SD) than the upregulated ones.
A comparison of the genes DE in response to drought under LD (LDD) with a previously published dataset (Maruyama et al. 2014) indicated that 3,568 genes (51%) were in common (Supporting Information Fig. S1). Among them, 94% of the genes were consistently up or downregulated in both datasets, indicating very good correlation and identifying a core set of genes responding to drought treatments independently of the experimental procedures used by different groups. Known stress markers were strongly upregulated in leaves subjected to drought stress independently of day length treatments. Conversely, in leaves exposed to SD, \(\text{Hd3a (FT-L2)}\) and \(\text{RFT1 (FT-L3)}\) transcripts were the most strongly induced in the entire dataset (Supporting Information Table S1).

Differentially expressed genes were then divided into categories based on their transcriptional behavior across all conditions (Table 1 and Supporting Information Table S1). Functional classes of DE genes were determined using gene ontology (GO) annotation. GO enrichment analysis was performed for some selected categories, depending on their possible relevance as points of convergence of drought and photoperiodic signals (Fig. 3). In particular, category A3.1 comprised genes whose expression was altered by drought stress but further modified if drought was applied under SD. Many specific GO terms enriched in this category were related to the metabolism of some amino acids and nucleotides. All genes annotated within these groups were downregulated under LDD but downregulation was attenuated under SDD. Category A4 comprised genes whose expression responded to drought under LD but was unaltered if drought was applied under SD. Many of the specific enriched GO terms were associated to light reactions, including chlorophyll metabolic process, photosynthesis light reaction, pigment biosynthetic process and thylakoid membrane organization. All such genes were downregulated in response to drought stress under LD. Additional terms including cytokinesis and regulation of organ growth, fatty acid metabolic process and phospholipid metabolism comprised genes that were mostly downregulated.
Category B2 grouped genes that were DE by SD and not by drought, and that showed expression levels similar to controls when treatments were combined. Category B2 contained enriched terms that were consistent with DNA organization, such as histone proteins, and all genes were upregulated by SD. Finally, category B3 comprised genes DE only when drought and SD treatments were combined. This category was enriched with genes related to protein synthesis such as pseudouridine synthesis and translation, and in each category genes were all or mostly upregulated. Terms related to protein phosphorylation were also included in this category and the corresponding genes were mostly downregulated.

Taken together, these data indicate that drought has a major impact on mRNA expression in leaves that respond to stress similarly under SD and LD. However, several genes belonging to specific metabolic processes respond to drought mainly or exclusively under specific day lengths.

**Induction of flowering by the photoperiodic pathway is antagonized by drought**

Exposure to drought stress strongly reduced expression of *Hd3a* and abolished that of *RFT1* in plants exposed to SD (Supporting Information Table S1), indicating that the flowering delay observed in stressed rice plants under inductive photoperiods might be caused by altered activity of the photoperiodic flowering network, and that drought and photoperiodic signals could converge on *Hd3a* and *RFT1* regulation.

To assess the effects of drought stress on expression of the florigens and to monitor some of their upstream regulators not highly expressed at the time of sampling for RNA-Seq, mRNA levels of genes central to the photoperiodic network were quantified during diurnal LD and SD time courses under drought stress conditions. Expression of *Hd3a* and *RFT1* was abolished in stressed leaves under both photoperiods, and during the entire time courses (Fig. 4g-4j). Similarly, a strong reduction of *Ehd1* transcript levels was observed in plants that
experienced water deprivation (Fig. 4e, 4f). The cycling amplitude of *Hd1* was reduced under SD (Fig. 4d), and abolished under LD (Fig. 4c). Under both photoperiods, transcripts were abundantly detected during the light phase under drought stress. Amplitude of *GI* mRNA expression was reduced under LD (Fig. 4a) but increased under SD (Fig. 4b) where transcription was higher during the light phase.

These data suggest that drought-mediated suppression of *Ehd1* expression could be responsible for decreased mRNA levels of florigenic genes and in turn caused by increased levels of *Hd1* during the light phase. Alternatively, drought-mediated signals could be dependent upon *Ehd1* but independent of *Hd1* and *GI*.

**The flowering delay quantitatively correlates with the length of drought stress**

Plants that experience drought stress during the floral induction delay flowering and fail to upregulate *Hd3a* and *RFT1*. Under field conditions, the intensity and duration of drought stress episodes can be variable. Whether delaying flowering can be an effective and flexible strategy to adjust the timing of the reproductive phase according to environmental stress was tested by measuring heading dates of plants that suffered drought for an increasing number of days. Distinct groups of plants were grown under LD for 4 weeks and then shifted to SD. The VWC% of the soil was progressively reduced in order to reach zero after 14, 16, 18 and 20 days after the shift to SD. A gradient of heading dates was observed, that was quantitatively dependent on the duration of drought (Fig. 5a). Quantification of *Hd3a*, *RFT1* and *Ehd1* transcripts was carried out at the end of and after the drought treatment. Plants that experienced drought started to accumulate *Hd3a*, *RFT1* and *Ehd1* transcripts as early as two days after the end of drought, showing sharp peaks of expression and similar dynamics (Fig. 5b-5f). These results indicate a linear correlation between the duration of drought, heading dates and the extent of induction of *Ehd1* and the florigens, and suggest that under drought
stress activation of the photoperiodic flowering system is arrested until environmental conditions become permissive.

**hd1 and osgi mutants delay flowering in response to drought stress**

Studies performed in Arabidopsis have suggested that functional *GI* is required to promote flowering under drought stress, whereas *CO* seems dispensable (Han et al. 2013; Riboni et al. 2013). Mutants in the rice photoperiodic flowering pathway were used to understand if the drought-mediated delay of flowering was dependent upon *Hd1* or *OsGI*.

Drought stress was applied to *hd1* mutants and NB during the floral transition and transcript abundance was quantified 2 hours after dawn in leaves exposed to 0, 6, 12 and 18 SD after the beginning of drought stress. Nipponbare plants showed increased *OsGI* and *Hd1* transcript levels under drought stress and reduced *Ehd1*, *Hd3a* and *RFT1* transcription (Fig. 6a, 6c, 6e, 6g, 6i). Patterns of expression of *hd1-1* mutants were qualitatively very similar to those measured in NB during developmental time courses (Fig. 6b, 6f, 6h, 6j). Moreover, *hd1-1* mutants headed later than NB and flowering was further delayed when plants were exposed to drought stress (Fig. 6k). Similar results were obtained using a second independent mutant allele (Supporting Information Fig. 2). These data indicate that *hd1* mutants respond to drought stress similarly to wild type and *Hd1* is unlikely to mediate drought stress signals that delay flowering.

To study the effects of mutations in *OsGI*, a novel allele was isolated in the NB background harbouring a *tos17* retrotransposon insertion in the fifth exon and that was referred to as *osg-i-3* (Fig. 7a). Expression of *OsGI* and *Hd3a* was undetectable in *osg-i-3* mutants upon shifting plants from LD to SD (Fig. 7c, 7d) and mutant plants flowered ~20 days later than NB (Fig. 7l), indicating that *osg-i-3* is likely to be a loss-of-function allele.
Since drought antagonizes induction of $Hd3a$ and $RFT1$ expression in leaves and in $osgi$-3 mutants transcription of these genes was extremely low for 48 days of exposure to SD, drought stress was prolonged until flowering of $osgi$-3 plants. Under such conditions, expression of $Hd1$ increased under drought stress in both NB and $osgi$-3 mutants (Fig. 7d, 7e), whereas peak expression of $Ehd1$ was strongly delayed consistent with the delay of heading dates (Fig. 7f, 7g). Expression of $Hd3a$ and $RFT1$ in $osgi$-3 was very low under drought stress similarly to control plants (Fig. 7i, 7k, confront scales with 7h and 7j), but eventually increased at 35 days after the shift. Similarly to $hd1$-1 mutants, flowering of $osgi$-3 plants was delayed by drought, indicating that the genetic effect of the mutation was additive to that of stress and that $OsGI$ does not integrate drought stress signals to regulate flowering, in contrast to what happens in Arabidopsis.

**DISCUSSION**

Drought stress has a major impact on rice yield, especially in rainfed areas where scarcity of water can represent a major constrain. The intensity, duration and timing of drought episodes can broadly vary depending on locations and years but the effects of drought on yield are particularly severe when water deficit occurs just prior flowering, because of damage to developing spikelets or to pollen grains (Fukai et al. 1999; Farooq et al. 2012). However, its unpredictable occurrence complicates the study of the effects of drought during specific phenological stages and different genotypes and environmental conditions can alter plant responses (Lanceras et al. 2004). Here, a single reference genotype grown under specific controlled conditions was used to study the effects of drought stress in rice plants grown under different day lengths, and a protocol was set up to synchronize the early stages of the floral transition with drought stress.
Previous data suggested that drought stress delays flowering in rice grown under field conditions, but the molecular mechanisms involved have never been elucidated (Fukai et al. 1999; Lanceras et al. 2004; Bocco et al. 2012). This study contributed to identify \textit{Hd3a}, \textit{RFT1} and \textit{Ehd1}, major integrators of light and photoperiodic signals, as integrators of drought stress responses as well.

\textbf{Modification of the drought stress transcriptome by changes in day length}

Previous studies have helped elucidate the regulatory networks that respond to water deficit and contributed to identify a core set of drought-responsive genes that help to cope with dehydration. Genes involved in the biosynthesis of osmoprotectants, including glucose, sucrose and proline, dehydrins, LEA proteins and several cytochrome P450 were all strongly activated in the SDD and LDD datasets, independently of day length. Similarly, photosynthesis related genes were mostly downregulated. However, several groups of genes showed expression profiles that were modulated differently by day length. Reduction of growth and cell division rates are typical responses of drought and category A4 was enriched with genes controlling the cell cycle that were downregulated by drought. However, this effect was observed under LD but not SD. Similarly, genes controlling phospholipid metabolism and some processes related to the function of the photosynthetic apparatus were downregulated under LD only. These results suggest that short days attenuate some of the detrimental effects of stress.

Category B2 represented another type of interaction, in which a SD-specific process, upregulation of several genes encoding core histone proteins, was antagonized by drought stress. This could indicate that under SD, overall levels of histones increase in leaves, as a consequence of chromatin remodeling in response to day length. Drought antagonizes this process by acting directly upon gene expression or indirectly on their chromatin status.
**Ehd1 and the florigens as integrators of drought and photoperiodic signals**

Drought causes transcriptional repression of *Ehd1*, *Hd3a* and *RFT1*. Among these, *Ehd1* is central in the flowering pathway and integrates light quality and photoperiodic signals (Brambilla & Fornara 2013). Its expression is induced by several upstream regulators including *Ehd2-4, Hd17* and *OsMADS50* (Matsubara 2008; Matsubara et al. 2011; Gao et al. 2013; Matsubara et al. 2012). Additionally, blue light signals gate *Ehd1* induction at dawn and this mechanism requires a functional *OsGI* protein (Itoh et al. 2010). Major repressors of flowering, including *Hd1, Ghd7, Ghd8* and *PRR37*, prevent *Ehd1* expression under non-inductive photoperiods (Gómez-Ariza et al. 2015; Xue et al. 2008; Yan et al. 2011, Gao et al. 2014).

Among the negative regulators of *Ehd1*, *Ghd7* has been shown to respond to abiotic stress signals at the transcriptional level (Weng et al. 2014). Young seedlings exposed to drought, heat and abscisic acid treatments rapidly reduced *Ghd7* mRNA levels (Weng et al. 2014). The datasets presented in this study are in agreement with these observations, indicating that *Ghd7* is repressed independently of day length. Additionally, *Ghd8* is also repressed by drought at least under SD. However, these dynamics would be compatible with increased, not reduced, *Ehd1* expression, suggesting that the interaction between photoperiodic flowering networks and drought signals is not mediated by *Ghd7* and *Ghd8*. Conversely, *Hd1* transcriptional levels were increased by drought and could possibly account for repression of *Ehd1*. Heading date assays performed under drought stress suggested that *Hd1* is not an integrator of drought stress signals, as the corresponding mutant plants responded to drought by delaying flowering, similarly to the wild type. Therefore, *Ehd1* likely integrates drought stress signals independently of its major upstream regulators. Whether *PRR37* can integrate drought stress signals to control *Ehd1* expression and flowering remains to be tested.
Drought-mediated repression of *Hd3a* and *RFT1* in leaves is likely the consequence of reduced *Ehd1* transcriptional levels (Doi et al. 2004; Zhao et al. 2015), although this study cannot exclude a direct effect of drought stress to repress transcription of florigenic loci.

**Drought escape strategies differ between rice and Arabidopsis**

Plants react to drought stress using different strategies, including drought escape, drought avoidance and drought tolerance (Kooyers et al. 2015b). During drought escape annual plants accelerate development and rapidly switch to the reproductive phase to complete their life cycle, before damage becomes irreversible. Several herbaceous plants employ escape strategies as *Boechera holboellii* (Knight et al. 2006), *Mimulus guttatus* (Kooyers et al. 2015a), *Helianthus anomalus* (Brouillette et al. 2014), *Panicum hallii* (Lowry et al. 2015), and *Arabidopsis thaliana* (Riboni et al. 2013). Also some crops as wheat and barley accelerate development under drought stress conditions (Mc Master & Wilhelm, 2003).

*Arabidopsis* has been instrumental to define some of the interactions between drought stress signals and components of the photoperiodic flowering network. Under LD conditions, plants grown under reduced water availability flower early and induce expression of *FLOWERING LOCUS T* (*FT*) and *TWIN SISTER OF FT* (*TSF*) to higher levels compared to normal watered controls (Riboni et al. 2013). Drought escape requires functional *GI*, because plants bearing mutations in the gene cannot flower early. Interestingly, *GI* mediates the escape response independently of other components required for LD flowering in Arabidopsis, including *FLAVIN BINDING KELCH REPEAT F BOX PROTEIN 1* (*FKF1*) and *CONSTANS* (*CO*) (Imaizumi et al. 2005; Fornara et al. 2009). However, functional *FT* and *TSF* are required, indicating that not all components of the flowering network are necessary for drought escape as they are for photoperiodic induction and that drought accelerates flowering possibly through a *GI-FT* direct pathway (Sawa & Kay 2011). A common feature underlying the
flowering response to drought of both rice and Arabidopsis is therefore the capacity to modulate expression of florigenic genes and to do so independently of some of their direct upstream regulators including \textit{Hd1} and \textit{CO}. However, as discussed above, the \textit{Ehd1}-dependent pathway, not shared by Arabidopsis, might have been recruited to mediate drought stress inputs into the flowering network.

A second remarkable similarity is that in Arabidopsis grown under non-inductive SD, flowering is delayed similarly to what happens in rice under the same photoperiodic conditions (Riboni et al. 2014), suggesting that the type of flowering response to drought might depend primarily on the day length under which plants are grown, to the extent that it can be reverted within the same species. This also raises the possibility that most crops typical of tropical areas could respond to water deficit by delaying flowering under inductive SD. Field experiments performed with maize and sorghum seem to corroborate this hypothesis, although dedicated studies are necessary (Abrecht & Carberry 1993; Farré & Faci, 2006; Craufurd & Peacock, 1993).

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**FIGURE LEGENDS**

**Figure 1.** Drought stress applied during the floral transition delays flowering of Nipponbare.

Volumetric Water Content (VWC%) curves of the soil are plotted and contrasted to the Relative Water Content (RWC) of leaves determined at 0, 6, 13 and 19 days after drought was applied to plants grown under continuous LD (a) or shifted to SD after 4 weeks of growth under LD (b). After 13 days of drought stress, plants showed rolled leaves under both LD (d) and SD (e), but not under normal watering (c). Quantification of mRNA expression of drought and photoperiod responsive genes including *OsDREB2A* (f), *OsNAC6* (g), *OsDhn1* (h) and *Hd3a* (i) at 0 and 13 days after the shift to SD (DAS). *Ubiquitin* was used to normalize expression values. Data are mean of three technical replicates and error bars indicate the standard deviation. Heading dates of drought-stressed and control plants are shown using box plots (j). Dashed lines and histograms indicate drought stress conditions, while continuous lines and filled histograms indicate watered controls. Red lines and blue lines indicate LD and SD conditions, respectively. Asterisks indicate statistically significant differences (P<0.0006 by Student’s t-test).

**Figure 2.** Global transcriptional profiling of drought stressed leaves under long and short photoperiods.

(a) Experimental set up: four-week-old plants grown under LD were shifted to SD applying drought (SDD) or normal water regimes (SDC), or maintained under LD applying drought (LDD) or normal water regimes (LDC). (b) Genome-wide transcriptional responses represented as circles and fragmented into 12 chromosomes. Peaks represent the log2 fold change of differentially up regulated (blue) and downregulated genes (red) under LDD (outermost circle), SDD (intermediate circle) and SDC (innermost circle) compared to the control condition LDC, and represented in their exact position on the genome. Numbers on
chromosome ideograms represent size in million bases. (c) Venn diagrams summarizing the number of differentially expressed genes among the conditions LDD, SDC, SDD compared to the control condition (LDC). Blue and red numbers among brackets indicate up and downregulated genes, respectively.

**Figure 3.** Gene Ontology enrichment of selected categories

Enriched specific GO terms are reported for selected categories. The y value refers to the log2 of the fold change ratio between enriched terms frequency and background frequency.

**Figure 4.** Drought alters diurnal expression patterns of genes controlling flowering.

Quantification of mRNA expression levels during diurnal time courses in NB grown under LD (a, c, e, g, i) and SD (b, d, f, h, j) conditions. Relative expression was measured for OsGI (a, b), Hdl (c, d), Ehd1 (e, f), Hd3a (g, h) and RFT1 (i, j). Dashed lines indicate patterns of drought stressed plants, while continuous lines indicate patterns in the normal watered controls. All samples were normalized using Ubiquitin. White and black bars on top of the graphs indicate the length of day and night periods, respectively. Numbers on the x axis indicate time from dawn (ZT, Zeitgeber).

**Figure 5.** The delay of flowering is quantitatively dependent on the length of drought stress.

(a) Heading dates of plants grown under varying drought stress lengths compared to controls. Quantification of Ehd1 (black line), Hd3a (red line) and RFT1 (blue line) mRNA expression levels determined after shifting 4-week-old plants to SD under normal watering conditions (b) or under drought stress applied for 14 (c), 16 (d), 18 (e) or 20 (f) days. Shaded areas on the graphs indicate the duration of the drought stress. One or two asterisks indicate P<0.008 and P<0.0006 respectively by Student’s t-test.
**Figure 6.** Drought stress delays flowering of *hd1* mutants.

Gene expression was measured during a time course on normal watered plants (black lines) and on drought stressed plants (dashed lines) in NB (a, c, e, g, i) and *hd1*-1 mutants (b, d, f, h, j). Expression profiles of *OsGI* (a, b), *Hd1* (c, d), *Ehd1* (e, f), *Hd3a* (g, h) and *RFT1* (i, j) were assessed. All samples were normalized using *Ubiquitin*. (k) Heading dates of NB and *hd1*-1 mutants grown under drought and normal watered regimes. Dotted boxes indicate the duration of drought stress. One or two asterisks indicate P< 0.008 and P<0.0006 respectively by Student’s t-test.

**Figure 7.** Drought stress delays flowering of *osgi* mutants.

A schematic representation of the *osgi-3* mutant allele (a). Black boxes indicate exons while black lines indicate introns. A triangle indicates the position of the *Tos17* insertion in the fifth exon of the gene. Quantification of *OsGI* (b) and *Hd3a* (c) mRNA expression in NB and in the *osgi*-3 mutant background after shifting plants to short days. Expression dynamics of *Hd1* (d, e), *Ehd1* (f, g), *Hd3a* (h, i) and *RFT1* (j, k) are plotted. (j) Heading dates of NB and *osgi-3* mutants exposed to drought stress or under normal watering. All the samples were normalized using *Ubiquitin*. Dotted areas indicate the duration of drought stress. One or two asterisks indicate P<0.008 and P<0.0006 respectively by Student’s t-test.

**Table 1.** List of categories of differentially expressed genes.

**Supporting information Figure 1.** Comparison between the SDD and Maruyama dataset.
Venn diagrams comparing the number of differentially expressed genes among the SDD dataset and the dataset presented by (Maruyama et al. 2014). Of the 3568 genes in common between the two dataset, 94% showed the same trend of expression.

**Supporting information Figure 2.** Drought stress delays flowering of *hd1*-2 mutants.

Gene expression was measured during a time course on normal watered plants (black lines) and on drought stressed plants (dashed lines) in NB (a, c, e, g, i) and *hd1*-2 mutants (b, d, f, h, j). Expression profiles of *OsGI* (a, b), *Hd1* (c, d), *Ehd1* (e, f), *Hd3a* (g, h) and *RFT1* (i, j) were assessed. All samples were normalized using *Ubiquitin*. (k) Heading dates of NB and *hd1*-2 mutants grown under drought and normal watered regimes. Dotted boxes indicate the duration of drought stress. Asterisks indicate P<0.0006 by Student’s t-test.

**Supporting information Table 1.** Lists of expressed and differentially expressed genes in all datasets. The first and second sheets include a detailed legend of the table.

**Supporting information Table 2.** List of primers used for quantification of gene expression.
Table 1. List of categories of differentially expressed genes.

<table>
<thead>
<tr>
<th>Category</th>
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<th>n. of genes</th>
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<td>Short Days (SD)</td>
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Legend:

- YES: Genes DE under the indicated condition
- NO: Genes not DE under the indicated condition