_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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26

27 **ABSTRACT**

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

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

50

51 **INTRODUCTION**

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

64 Plant responses to dehydration cause changes at the physiological, morphological and
65 molecular level, including altered transcription patterns of many genes (Shinozaki &
66 Yamaguchi-Shinozaki, 2007, Rabbani et al. 2003; Ray et al. 2011; Maruyama et al. 2012).
67 Drought responsive genes can be divided into two groups based on their involvement in
68 protecting cells against environmental stress or in regulating genes that transduce stress
69 response signals (Ingram et al. 1996; Shinozaki & Yamaguchi-Shinozaki, 2000; Minh-Thu et
70 al. 2013). The first group includes water channel proteins, lipid desaturases and enzymes
71 catalyzing the biosynthesis of osmoprotectants such as glycerol, mannitol, sucrose and proline
72 (Ray et al. 2011; Minh-Thu et al. 2013). The second comprises master regulatory proteins
73 including transcription factors, protein kinases and chromatin remodeling factors (Agarwal et
74 al. 2006; Nakashima et al. 2009; Yu et al. 2013; Han & Wagner 2014).

75 Increasing evidences document that drought also impacts on the flowering process in diverse
76 species (Sherrard & Maherali, 2006; Bocco et al. 2012; Franks, 2011; Bernal et al. 2011; Ivey
77 & Carr, 2012; Kobayashi et al. 2013; Riboni et al. 2013). Several plants, including
78 *Arabidopsis thaliana*, wheat and barley adopt a drought escape strategy whereby water
79 deprivation rapidly induces flowering and seed set, in order to complete the life cycle before
80 stress conditions become lethal (Mc Master & Wilhelm, 2003; Sherrard & Maherali, 2006;
81 Franks et al. 2007; Bernal et al. 2011; Franks, 2011). Conversely, other species respond to
82 drought by delaying flowering, eventually resuming it as environmental stress is over.
83 Therefore, regulatory connections exist between the drought response and floral induction
84 pathways. It is however unclear if they are shared between species that deploy different
85 strategies to cope with drought stress.
86 Flowering of Arabidopsis is rapidly induced when plants are exposed to long days (LD), and a
87 genetic cascade comprising the *GIGANTEA* (*GI*), *FLAVIN BINDING KELCH REPEAT F-*
88 *BOX PROTEIN 1* (*FKF1*), *CONSTANS* (*CO*) and *CYCLING DOF FACTOR* genes (*CDFs*)
89 activates the transcription of *FLOWERING LOCUS T* (*FT*) and *TWIN SISTER OF FT* (*TSF*),
90 components of the florigenic signal (Andrés & Coupland, 2012). Drought applied under LD
91 triggers a drought escape response that is not observed under short days (SD) or in plants
92 where *GI* or *FT* and *TSF* are mutated (Riboni et al. 2013).
93 Flowering in rice (also called heading) is activated under short photoperiods, whereas long
94 days have a repressive effect on the floral transition. This type of photoperiodic response,
95 despite being opposite from that of Arabidopsis, depends on a genetic cascade that shares the
96 same components (Shrestha et al. 2014). Under SD, the OsGI protein induces expression of
97 *HEADING DATE 1* (*Hd1*), a homolog of *CO*, that in turn activates the transcription of
98 *HEADING DATE 3A* (*Hd3a*) and *RICE FLOWERING LOCUS T 1* (*RFT1*), homologs of *FT*
99 (Hayama et al. 2003; Komiya et al. 2008). Rice evolved a parallel inductive pathway that can

100 promote expression of *Hd3a* and *RFT1*, and is dependent on the function of *EARLY*
101 *HEADING DATE 1* (*Ehd1*) (Doi et al. 2004). Under non-inductive LD, *Hd1* represses the
102 transcription of *Ehd1*, *Hd3a* and *RFT1*, delaying the floral transition (Gómez-Ariza et al.
103 2015). The dual role of *Hd1* as SD activator and LD repressor of the flowering process is a
104 feature not shared by Arabidopsis *CO*.
105 Heading of rice plants is delayed upon exposure to drought. The effect has been reported in
106 different varieties and upon stressing at different developmental stages (Fisher & Fukai 2003;
107 Ji et al. 2005; Bocco et al. 2012). Despite the key importance of correct heading dates for
108 reproductive success, it is currently unclear (i) how rice plants respond to water deprivation
109 during photoperiodic induction and (ii) whether day length affects the response to abiotic
110 stresses. Finally, it is not clear if components of the photoperiodic network are involved in the
111 flowering response of rice plants under drought stress, and whether the conclusions obtained
112 using Arabidopsis as model system constitute a widely applicable frame for studying the
113 interaction between drought and the flowering process in monocot species adapted to SD.
114 In this study, we explored the effects of drought stress applied to rice plants grown under
115 specific photoperiods and during floral commitment, when plants switch from vegetative to
116 reproductive growth. Transcriptional responses were assessed at the genome-wide scale and
117 genes differentially responding to drought depending on the photoperiod were identified.
118 *Ehd1*, *Hd3a* and *RFT1* were identified as points of convergence of flowering and drought
119 signals in rice. However, the drought response was not altered in *osgi* mutants, underlying
120 distinct responses between rice and Arabidopsis.
121

122 MATERIALS AND METHODS

123 Plant material and growth conditions

124 Nipponbare (NB) seeds were used for all the experiments. Plants were grown in Conviron
125 PGR15 chambers set on temperature/relative humidity cycles of 28ºC/80% during the day and
126 24ºC/90% during the night. Light was provided by fluorescent tubes and metal halide bulbs
127 (intensity of ~450µE/m^{-2}sec^{-1}). The mutant line AB156681 corresponding to the *OsGI* locus
128 and named *osgi-3* was obtained from the National Institute of Agrobiological Sciences of
129 Japan (https://tos.nias.affrc.go.jp/). Mutant plants were genotyped using primers specific for
130 *tos17* 5'-GTACTGTATAGTTGGCCCATGTCC-3' and *OsGI* 5'-
131 CCTGCGTTCTGCTCACATACTTC-3'. The *hd1-1* and *hd1-2* mutant alleles were
132 previously described (Gómez-Ariza et al. 2015). Heading date measurements were obtained
133 from at least 15 plants per genotype.
134

135 **Drought stress assays**

136 One hundred seeds of NB were planted on soil in 10L square boxes and plants were grown for
137 4 weeks under LD conditions and a normal water regime. After 4 weeks, half of the pots were
138 moved to SD. Under each photoperiod, half of the pots were watered normally (control),
139 while the remaining half was subjected to drought stress. Soil water content was monitored
140 every hour using moisture sensors (WaterScout SM 100 Soil Moisture Sensor® and
141 WatchDog A-Series Loggers®). The relative water content (RWC) was measured according
142 to (Baldoni et al. 2013). The analysis of covariance (ANCOVA) was applied to RWC data
143 using plant height as covariate and α=0.05. Finally, expression of drought responsive genes
144 *DEHYDRATION-RESPONSIVE ELEMENT BINDING PROTEIN 2A* (*OsDREB2A*), *Dehydrin*
145 *1* (*OsDhn1*) and *OsNAC6* was quantified in each experiment using a Mastercycler Realplex^2
146 (Eppendorf).

147 For LD and SD diurnal time course experiments, seeds were planted in two distinct 10L
148 square pots and watered normally or exposed to drought stress. Samples were collected after 8
149 or 6 weeks from plants grown under LD and SD, respectively.

150

151 **RNA extraction and quantification of mRNA expression**

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

160

161 **RNA sequencing and data processing**

162 Total RNA was extracted using the NucleoSpin® RNA Plant (Macherey-Nagel) kit and total
163 RNA was treated and quantified as above. Sequencing was performed at BGI Tech Solutions
164 (Hong Kong) using an Illumina HiSeq 2000. RNA-seq yielded 24 to 37 millions of cleaned 50
165 bp single reads (Supporting Information Table S1). Quality of raw data was checked using the
166 FastQC tool for high throughput sequence data
167 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). About 98% of the cleaned reads
168 were aligned against the *O. sativa* Japonica group cultivar Nipponbare genome version 7.0
169 (http://rice.plantbiology.msu.edu/) using bowtie2 and TopHat2 (Langmead & Salzberg 2012,
170 Kim et al. 2013). About 90% of the reads were uniquely mapped and counted by HTSeq
171 (Anders et al. 2015) and subsequently used for gene-level differential expression analysis

172 using the R software (version 3.1.2) for statistical computing and edgeR package version
173 3.6.8 from bioconductor (Robinson et al. 2010, Gentleman et al. 2004). For subsequent
174 analyses, only features with more than 1 read per million in at least 3 samples were retained,
175 for a total of 23,672 genes expressed across all conditions. Differentially expressed (DE)
176 genes were called using a 1% false discovery rate (FDR) control and filtered for log2 fold
177 change (FC) values below or above 1.5 yielding 8,359 DE genes. Gene Ontology enrichments
178 were performed using PANTHER (Mi H. et al. 2013) from the GOC website
179 (http://geneontology.org). Circular data visualization was obtained with the circus software
180 package (Krzywinski et al. 2009).

181

182 **RESULTS**

183 **Synchronizing the floral transition and drought stress**

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

196 values had reached pre-drought levels (Fig. 1a, 1b). All plants survived and were able to
197 complete their life cycle.
198 The molecular response to drought was monitored by quantifying the mRNA expression
199 levels of drought-responsive genes, including *OsDREB2A*, *OsNAC6* and *OsDhn1* (Dubouzet
200 et al. 2003; Nakashima et al. 2007; Lee et al. 2005). All genes showed a marked increase of
201 expression after 13 days of drought compared to control plants (Fig. 1f-1h). Response to the
202 SD treatment was monitored by quantifying *Hd3a* expression that was increased 13 days after
203 the shift to SD (Fig. 1i). Finally, heading dates were scored and a statistically significant and
204 reproducible delay was observed in plants exposed to drought stress under SD conditions
205 (Fig. 1j). After recovery, plants set seeds normally and fertility was similar between treated
206 and untreated plants (data not shown). In conclusion, this protocol provides a reliable method
207 to assess the interaction between drought stress and photoperiodic induction in soil-grown
208 plants.
209

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

211 The global transcriptional effects of drought stress were monitored in leaves using RNA-
212 sequencing. RNA was extracted from leaves subjected to dual treatments according to the
213 scheme of Fig. 2a. Differentially expressed genes were uniformly distributed along the
214 chromosomes and most of them were induced or repressed independently of the photoperiod
215 with 5,937 genes (71%) in common between samples subjected to drought stress under SD
216 and LD treatments (Fig. 2b, 2c). The number of genes differentially expressed in response to
217 drought was overall greater (7,854) than the number of genes controlled by day length
218 (1,645), suggesting a major impact of water deficit on global transcription in the leaves
219 (Supporting Information Table S1). The proportion of genes downregulated by drought was
220 always higher (59% under LD and 57% under SD) than the upregulated ones.

221 A comparison of the genes DE in response to drought under LD (LDD) with a previously
222 published dataset (Maruyama et al. 2014) indicated that 3,568 genes (51%) were in common
223 (Supporting Information Fig. S1). Among them, 94% of the genes were consistently up or
224 downregulated in both datasets, indicating very good correlation and identifying a core set of
225 genes responding to drought treatments independently of the experimental procedures used by
226 different groups. Known stress markers were strongly upregulated in leaves subjected to
227 drought stress independently of day length treatments. Conversely, in leaves exposed to SD,
228 *Hd3a* (*FT-L2*) and *RFT1* (*FT-L3*) transcripts were the most strongly induced in the entire
229 dataset (Supporting Information Table S1).
230 Differentially expressed genes were then divided into categories based on their transcriptional
231 behavior across all conditions (Table 1 and Supporting Information Table S1). Functional
232 classes of DE genes were determined using gene ontology (GO) annotation. GO enrichment
233 analysis was performed for some selected categories, depending on their possible relevance as
234 points of convergence of drought and photoperiodic signals (Fig. 3). In particular, category
235 A3.1 comprised genes whose expression was altered by drought stress but further modified if
236 drought was applied under SD. Many specific GO terms enriched in this category were
237 related to the metabolism of some amino acids and nucleotides. All genes annotated within
238 these groups were downregulated under LDD but downregulation was attenuated under SDD.
239 Category A4 comprised genes whose expression responded to drought under LD but was
240 unaltered if drought was applied under SD. Many of the specific enriched GO terms were
241 associated to light reactions, including chlorophyll metabolic process, photosynthesis light
242 reaction, pigment biosynthetic process and thylakoid membrane organization. All such genes
243 were downregulated in response to drought stress under LD. Additional terms including
244 cytokinesis and regulation of organ growth, fatty acid metabolic process and phospholipid
245 metabolism comprised genes that were mostly downregulated.

246 Category B2 grouped genes that were DE by SD and not by drought, and that showed
247 expression levels similar to controls when treatments were combined. Category B2 contained
248 enriched terms that were consistent with DNA organization, such as histone proteins, and all
249 genes were upregulated by SD. Finally, category B3 comprised genes DE only when drought
250 and SD treatments were combined. This category was enriched with genes related to protein
251 synthesis such as pseudouridine synthesis and translation, and in each category genes were all
252 or mostly upregulated. Terms related to protein phosphorylation were also included in this
253 category and the corresponding genes were mostly downregulated.
254 Taken together, these data indicate that drought has a major impact on mRNA expression in
255 leaves that respond to stress similarly under SD and LD. However, several genes belonging to
256 specific metabolic processes respond to drought mainly or exclusively under specific day
257 lengths.
258

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

260 Exposure to drought stress strongly reduced expression of *Hd3a* and abolished that of *RFT1*
261 in plants exposed to SD (Supporting Information Table S1), indicating that the flowering
262 delay observed in stressed rice plants under inductive photoperiods might be caused by
263 altered activity of the photoperiodic flowering network, and that drought and photoperiodic
264 signals could converge on *Hd3a* and *RFT1* regulation.
265 To assess the effects of drought stress on expression of the florigens and to monitor some of
266 their upstream regulators not highly expressed at the time of sampling for RNA-Seq, mRNA
267 levels of genes central to the photoperiodic network were quantified during diurnal LD and
268 SD time courses under drought stress conditions. Expression of *Hd3a* and *RFT1* was
269 abolished in stressed leaves under both photoperiods, and during the entire time courses (Fig.
270 4g-4j). Similarly, a strong reduction of *Ehd1* transcript levels was observed in plants that

271 experienced water deprivation (Fig. 4e, 4f). The cycling amplitude of *Hd1* was reduced under
272 SD (Fig. 4d), and abolished under LD (Fig. 4c). Under both photoperiods, transcripts were
273 abundantly detected during the light phase under drought stress. Amplitude of *GI* mRNA
274 expression was reduced under LD (Fig. 4a) but increased under SD (Fig. 4b) where
275 transcription was higher during the light phase.
276 These data suggest that drought-mediated suppression of *Ehd1* expression could be
277 responsible for decreased mRNA levels of florigenic genes and in turn caused by increased
278 levels of *Hd1* during the light phase. Alternatively, drought-mediated signals could be
279 dependent upon *Ehd1* but independent of *Hd1* and *GI*.
280

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

282 Plants that experience drought stress during the floral induction delay flowering and fail to
283 upregulate *Hd3a* and *RFT1*. Under field conditions, the intensity and duration of drought
284 stress episodes can be variable. Whether delaying flowering can be an effective and flexible
285 strategy to adjust the timing of the reproductive phase according to environmental stress was
286 tested by measuring heading dates of plants that suffered drought for an increasing number of
287 days. Distinct groups of plants were grown under LD for 4 weeks and then shifted to SD. The
288 VWC% of the soil was progressively reduced in order to reach zero after 14, 16, 18 and 20
289 days after the shift to SD. A gradient of heading dates was observed, that was quantitatively
290 dependent on the duration of drought (Fig. 5a). Quantification of *Hd3a*, *RFT1* and *Ehd1*
291 transcripts was carried out at the end of and after the drought treatment. Plants that
292 experienced drought started to accumulate *Hd3a*, *RFT1* and *Ehd1* transcripts as early as two
293 days after the end of drought, showing sharp peaks of expression and similar dynamics (Fig.
294 5b-5f). These results indicate a linear correlation between the duration of drought, heading
295 dates and the extent of induction of *Ehd1* and the florigens, and suggest that under drought

296 stress activation of the photoperiodic flowering system is arrested until environmental
297 conditions become permissive.
298
299 ***hd1* and *osgi* mutants delay flowering in response to drought stress**
300 Studies performed in Arabidopsis have suggested that functional *GI* is required to promote
301 flowering under drought stress, whereas *CO* seems dispensable (Han et al. 2013; Riboni et al.
302 2013). Mutants in the rice photoperiodic flowering pathway were used to understand if the
303 drought-mediated delay of flowering was dependent upon *Hd1* or *OsGI*.
304 Drought stress was applied to *hd1* mutants and NB during the floral transition and transcript
305 abundance was quantified 2 hours after dawn in leaves exposed to 0, 6, 12 and 18 SD after the
306 beginning of drought stress. Nipponbare plants showed increased *OsGI* and *Hd1* transcript
307 levels under drought stress and reduced *Ehd1*, *Hd3a* and *RFT1* transcription (Fig. 6a, 6c, 6e,
308 6g, 6i). Patterns of expression of *hd1-1* mutants were qualitatively very similar to those
309 measured in NB during developmental time courses (Fig. 6b, 6f, 6h, 6j). Moreover, *hd1-1*
310 mutants headed later than NB and flowering was further delayed when plants were exposed to
311 drought stress (Fig. 6k). Similar results were obtained using a second independent mutant
312 allele (Supporting Information Fig. 2). These data indicate that *hd1* mutants respond to
313 drought stress similarly to wild type and *Hd1* is unlikely to mediate drought stress signals that
314 delay flowering.
315 To study the effects of mutations in *OsGI*, a novel allele was isolated in the NB background
316 harbouring a *tos17* retrotransposon insertion in the fifth exon and that was referred to as *osgi-*
317 *3* (Fig. 7a). Expression of *OsGI* and *Hd3a* was undetectable in *osgi-3* mutants upon shifting
318 plants from LD to SD (Fig. 7c, 7d) and mutant plants flowered ~20 days later than NB (Fig.
319 7l), indicating that *osgi-3* is likely to be a loss-of-function allele.

320 Since drought antagonizes induction of *Hd3a* and *RFT1* expression in leaves and in *osgi-3*
321 mutants transcription of these genes was extremely low for 48 days of exposure to SD,
322 drought stress was prolonged until flowering of *osgi-3* plants. Under such conditions,
323 expression of *Hd1* increased under drought stress in both NB and *osgi-3* mutants (Fig. 7d, 7e),
324 whereas peak expression of *Ehd1* was strongly delayed consistent with the delay of heading
325 dates (Fig. 7f, 7g). Expression of *Hd3a* and *RFT1* in *osgi-3* was very low under drought stress
326 similarly to control plants (Fig. 7i, 7k, confront scales with 7h and 7j), but eventually
327 increased at 35 days after the shift. Similarly to *hd1-1* mutants, flowering of *osgi-3* plants was
328 delayed by drought, indicating that the genetic effect of the mutation was additive to that of
329 stress and that *OsGI* does not integrate drought stress signals to regulate flowering, in contrast
330 to what happens in Arabidopsis.
331
332 **DISCUSSION**
333 Drought stress has a major impact on rice yield, especially in rainfed areas where scarcity of
334 water can represent a major constrain. The intensity, duration and timing of drought episodes
335 can broadly vary depending on locations and years but the effects of drought on yield are
336 particularly severe when water deficit occurs just prior flowering, because of damage to
337 developing spikelets or to pollen grains (Fukai et al. 1999; Farooq et al. 2012). However, its
338 unpredictable occurrence complicates the study of the effects of drought during specific
339 phenological stages and different genotypes and environmental conditions can alter plant
340 responses (Lanceras et al. 2004). Here, a single reference genotype grown under specific
341 controlled conditions was used to study the effects of drought stress in rice plants grown
342 under different day lengths, and a protocol was set up to synchronize the early stages of the
343 floral transition with drought stress.

344 Previous data suggested that drought stress delays flowering in rice grown under field
345 conditions, but the molecular mechanisms involved have never been elucidated (Fukai et al.
346 1999; Lanceras et al. 2004; Bocco et al. 2012). This study contributed to identify *Hd3a*, *RFT1*
347 and *Ehd1*, major integrators of light and photoperiodic signals, as integrators of drought stress
348 responses as well.

349

350 **Modification of the drought stress transcriptome by changes in day length**

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

364 Category B2 represented another type of interaction, in which a SD-specific process,
365 upregulation of several genes encoding core histone proteins, was antagonized by drought
366 stress. This could indicate that under SD, overall levels of histones increase in leaves, as a
367 consequence of chromatin remodeling in response to day length. Drought antagonizes this
368 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.

394 Drought-mediated repression of *Hd3a* and *RFT1* in leaves is likely the consequence of
395 reduced *Ehd1* transcriptional levels (Doi et al. 2004; Zhao et al. 2015), although this study
396 cannot exclude a direct effect of drought stress to repress transcription of florigenic loci.
397
398 **Drought escape strategies differ between rice and Arabidopsis**
399 Plants react to drought stress using different strategies, including drought escape, drought
400 avoidance and drought tolerance (Kooyers et al. 2015b). During drought escape annual plants
401 accelerate development and rapidly switch to the reproductive phase to complete their life
402 cycle, before damage becomes irreversible. Several herbaceous plants employ escape
403 strategies as *Boechera holboellii* (Knight et al. 2006), *Mimulus guttatus* (Kooyers et al.
404 2015a), *Helianthus anomalus* (Brouillette et al. 2014), *Panicum hallii* (Lowry et al. 2015),
405 and *Arabidopsis thaliana* (Riboni et al. 2013). Also some crops as wheat and barley accelerate
406 development under drought stress conditions (Mc Master & Wilhelm, 2003).
407 Arabidopsis has been instrumental to define some of the interactions between drought stress
408 signals and components of the photoperiodic flowering network. Under LD conditions, plants
409 grown under reduced water availability flower early and induce expression of *FLOWERING*
410 *LOCUS T* (*FT*) and *TWIN SISTER OF FT* (*TSF*) to higher levels compared to normal watered
411 controls (Riboni et al. 2013). Drought escape requires functional *GI*, because plants bearing
412 mutations in the gene cannot flower early. Interestingly, *GI* mediates the escape response
413 independently of other components required for LD flowering in Arabidopsis, including
414 *FLAVIN BINDING KELCH REPEAT F BOX PROTEIN 1* (*FKF1*) and *CONSTANS* (*CO*)
415 (Imaizumi et al. 2005; Fornara et al. 2009). However, functional *FT* and *TSF* are required,
416 indicating that not all components of the flowering network are necessary for drought escape
417 as they are for photoperiodic induction and that drought accelerates flowering possibly
418 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 *Hd1* and *CO*. However, as discussed above, the *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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668 **FIGURE LEGENDS**

669 **Figure 1**. Drought stress applied during the floral transition delays flowering of Nipponbare.
670 Volumetric Water Content (VWC%) curves of the soil are plotted and contrasted to the
671 Relative Water Content (RWC) of leaves determined at 0, 6, 13 and 19 days after drought was
672 applied to plants grown under continuous LD (a) or shifted to SD after 4 weeks of growth
673 under LD (b). After 13 days of drought stress, plants showed rolled leaves under both LD (d)
674 and SD (e), but not under normal watering (c). Quantification of mRNA expression of
675 drought and photoperiod responsive genes including *OsDREB2A* (f), *OsNAC6* (g), *OsDhn1*
676 (h) and *Hd3a* (i) at 0 and 13 days after the shift to SD (DAS). *Ubiquitin* was used to
677 normalize expression values. Data are mean of three technical replicates and error bars
678 indicate the standard deviation. Heading dates of drought-stressed and control plants are
679 shown using box plots (j). Dashed lines and histograms indicate drought stress conditions,
680 while continuous lines and filled histograms indicate watered controls. Red lines and blue
681 lines indicate LD and SD conditions, respectively. Asterisks indicate statistically significant
682 differences (P<0,0006 by Student's t-test).

683

684 **Figure 2**. Global transcriptional profiling of drought stressed leaves under long and short
685 photoperiods.
686 (a) Experimental set up: four-week-old plants grown under LD were shifted to SD applying
687 drought (SDD) or normal water regimes (SDC), or maintained under LD applying drought
688 (LDD) or normal water regimes (LDC). (b) Genome-wide transcriptional responses
689 represented as circles and fragmented into 12 chromosomes. Peaks represent the log2 fold
690 change of differentially up regulated (blue) and downregulated genes (red) under LDD
691 (outermost circle), SDD (intermediate circle) and SDC (innermost circle) compared to the
692 control condition LDC, and represented in their exact position on the genome. Numbers on

693 chromosome ideograms represent size in million bases. (c) Venn diagrams summarizing the
694 number of differentially expressed genes among the conditions LDD, SDC, SDD compared to
695 the control condition (LDC). Blue and red numbers among brackets indicate up and
696 downregulated genes, respectively.

697

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

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

701

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

703 Quantification of mRNA expression levels during diurnal time courses in NB grown under
704 LD (a, c, e, g, i) and SD (b, d, f, h, j) conditions. Relative expression was measured for *OsGI*
705 (a, b), *Hd1* (c, d), *Ehd1* (e, f), *Hd3a* (g, h) and *RFT1* (i, j). Dashed lines indicate patterns of
706 drought stressed plants, while continuous lines indicate patterns in the normal watered
707 controls. All samples were normalized using *Ubiquitin.* White and black bars on top of the
708 graphs indicate the length of day and night periods, respectively. Numbers on the x axis
709 indicate time from dawn (ZT, *Zeitgeber*).

710

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

712 (a) Heading dates of plants grown under varying drought stress lengths compared to controls.
713 Quantification of *Ehd1* (black line), *Hd3a* (red line) and *RFT1* (blue line) mRNA expression
714 levels determined after shifting 4-week-old plants to SD under normal watering conditions (b)
715 or under drought stress applied for 14 (c), 16 (d), 18 (e) or 20 (f) days. Shaded areas on the
716 graphs indicate the duration of the drought stress. One or two asteriscs indicate $P< 0,008$ and
717 $P<0,0006$ respectively by Student’s t-test.

718

719 **Figure 6**. Drought stress delays flowering of *hd1* mutants.

720 Gene expression was measured during a time course on normal watered plants (black lines)
721 and on drought stressed plants (dashed lines) in NB (a, c, e, g, i) and *hd1-1* mutants (b, d, f, h,
722 j). Expression profiles of *OsGI* (a, b), *Hd1* (c, d), *Ehd1* (e, f), *Hd3a* (g, h) and *RFT1* (i, j) were
723 assessed. All samples were normalized using *Ubiquitin*. (k) Heading dates of NB and *hd1-1*
724 mutants grown under drought and normal watered regimes. Dotted boxes indicate the duration
725 of drought stress. One or two asteriscs indicate P< 0,008 and P<0,0006 respectively by
726 Student's t-test.

727

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

729 A schematic representation of the *osgi-3* mutant allele (a). Black boxes indicate exons while
730 black lines indicate introns. A triangle indicates the position of the *Tos17* insertion in the fifth
731 exon of the gene. Quantification of *OsGI* (b) and *Hd3a* (c) mRNA expression in NB and in
732 the *osgi-3* mutant background after shifting plants to short days. Expression dynamics of *Hd1*
733 (d, e), *Ehd1* (f, g), *Hd3a* (h, i) and *RFT1* (j, k) are plotted. (j) Heading dates of NB and *osgi-3*
734 mutants exposed to drought stress or under normal watering. All the samples were normalized
735 using *Ubiquitin*. Dotted areas indicate the duration of drought stress. One or two asterisks
736 indicate P<0,008 and P<0,0006 respectively by Student's t-test.

737

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

739

740 **Supporting information Figure 1**. Comparison between the SDD and Maruyama dataset.

741 Venn diagrams comparing the number of differentially expressed genes among the SDD
742 dataset and the dataset presented by (Maruyama et al. 2014). Of the 3568 genes in common
743 between the two dataset, 94% showed the same trend of expression.
744
745 **Supporting information Figure 2.** Drought stress delays flowering of *hd1-2* mutants.
746 Gene expression was measured during a time course on normal watered plants (black lines)
747 and on drought stressed plants (dashed lines) in NB (a, c, e, g, i) and *hd1-2* mutants (b, d, f, h,
748 j). Expression profiles of *OsGI* (a, b), *Hd1* (c, d), *Ehd1* (e, f), *Hd3a* (g, h) and *RFT1* (i, j) were
749 assessed. All samples were normalized using *Ubiquitin*. (k) Heading dates of NB and *hd1-2*
750 mutants grown under drought and normal watered regimes. Dotted boxes indicate the duration
751 of drought stress. Asterisks indicate P<0,0006 by Student's t-test.
752
753 **Supporting information Table 1.** Lists of expressed and differentially expressed genes in all
754 datasets. The first and second sheets include a detailed legend of the table.
755
756 **Supporting information Table 2.** List of primers used for quantification of gene expression.
757

Table 1. List of categories of differentially expressed genes.

Category	Expression modified under			n. of genes	Transcriptional behaviour
	Drought stress (D)	**Short Days (SD)**	**Drought stress and Short Days (SDD)**		
A1	YES	YES	YES	924	Genes DE under D and SD in which expression levels under SDD are similar to those under D or SD alone
A2	YES	YES	NO	107	Genes DE under D and SD in which SDD antagonizes the effects of each treatment alone
A3	YES	NO	YES	4913	Genes DE under drought stress
A3.1	YES	NO	YES and DE compared to LDD	100	Genes DE under D in which SDD antagonizes or enhances the effects of D
A4	YES	NO	NO	954	Genes DE under D and in which the SD treatment abolishes the effects of D
B1	NO	YES	YES	109	Genes DE under short days
B2	NO	YES	NO	505	Genes DE under SD and in which the D treatment abolishes the effect of SD
B3	NO	NO	YES	747	Genes DE only when D is applied under SD