Genotypic response to water deficit applied at reproductive stage in rice: is the response unique across two contrasted climates?

Isabela Pereira de Lima (isabelailima@yahoo.com.br)
Universidade Federal de Lavras Departamento de Agricultura https://orcid.org/0000-0002-6090-682X

Tanguy Lafarge
CIRAD

Adriano Pereira Castro
Embrapa Arroz e Feijão

Sandrine Roques
CIRAD

Armelle Soutiras
CIRAD

Anne Clement-Vidal
CIRAD

Flávia Barbosa Silva Botelho
Universidade Federal de Lavras Departamento de Agricultura

Marcel de Raissac
CIRAD

Original article

Keywords: Oryza sativa, fine phenotyping, reproductive phase, drought tolerance, yield components, flag leaf width

Posted Date: April 2nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-20289/v1

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Isabela Pereira de Lima[1], Tanguy Lafarge[2], Adriano Pereira de Castro[3], Sandrine Roques[2], Armelle Soutiras[2], Anne Clément-Vidal[2], Flávia Barbosa Silva Botelho[1], Marcel de Raïssac[2]

[1]Universidade Federal de Lavras, Departamento de Agricultura. Campus Universitário, Lavras, MG, 37200-900, Brazil

[2]CIRAD, UMR AGAP, F-34398 Montpellier, France

AGAP, CIRAD, INRA, Montpellier SupAgro, University Montpellier, France

[3]Embrapa Arroz e Feijão, Rodovia GO-462, km 12, Santo Antônio de Goiás, GO, 75375-000, Brazil

Corresponding author:

Isabela Pereira de Lima, Universidade Federal de Lavras, Departamento de Agricultura. Campus Universitário, MG, 37200-900, Brazil. *isabelailima@yahoo.com.br*

Abstract

Background: Rice crop is known as very sensitive to water deficit, especially during the reproductive phase when growth of vegetative organs and formation of spikelets are concomitant. The present study questioned whether the maintenance of vegetative organ expansion during a water deficit at the reproductive phase affects the reproductive structure organogenesis, and if relevant traits for adaptation can be identified. To answer these queries, the response to a same reproductive water deficit of six contrasted japonica genotypes has been analyzed under two contrasted climates differing in incident radiation, air hygrometry and temperature (greenhouse in Brazil, Sitis, and growth chamber in France, GC).

Results: Under irrigation, plant growth rate was reduced in GC while crop duration was extended: the trade-offs resulted in similar plant height and biomass. From a method able to determine *a posteriori* the date of panicle initiation (PI), elementary processes were positioned in time, allowing to evaluate how much each process was affected, despite the diversity in phenology across genotypes. Elongation rate decreased with water deficit and was highly associated with an increase in soluble sugars in stem and flag leaf in both experiments, while starch was reduced in Sitis and negligible in GC. This unique response, however, induced a different effect across experiments on branch and spikelet formation. In short, in both experiments, maintenance of spikelet number was highly associated with maintenance of flag leaf and internode width. All variables, including branch and spikelet number, were highly affected with IAC 25 in both experiments, while variations were non-significant with Cirad 409. In addition, some other genotypes expressed a differential response between Sitis and GC, conveying a specific sensitivity to low radiation or to high air temperature.

Conclusions: This study highlighted the importance of finely mapping in time elementary growth processes, thanks to PI date determination, in order to detect key traits of adaptation to abiotic stress through phenotyping across genotypes of diverse phenology. The maintenance of flag leaf width or internode diameter under a mild water deficit was highlighted here as a trait associated with the maintenance of yield components.

Keywords: *Oryza sativa*, fine phenotyping, reproductive phase, drought tolerance, yield components, flag leaf width.

Background

Rice (*Oryza sativa* L.) is known to be particularly sensitive to soil water deficit because of its semi-aquatic origin (Kumar et al. 2014). Drought is therefore considered as the most important constraint reducing yield in rainfed areas (Kumar et al. 2008; Serraj et al. 2009). Water deficit may impact rice yield components according to its timing, duration and intensity: at vegetative stage, it can affect development rate, plant height, leaf area and tillering. During the reproductive phase, it mainly affects panicle branching, spikelet formation and pollen viability. After flowering, it can affect grain setting and filling and their resulting grain number and weight. Literature converges in pinpointing the reproductive phase as the most sensitive. According to Matsushima (1966), the period of time between minus eleven and minus three days before heading is the most sensitive period by its impact on final yield. Similarly, comparing the effect of water deficit at different growth periods, Lilley and Fukai (1994) and Boonjung and Fukai (1996a) demonstrated that drought has non-significant or small impact on subsequent development and grain yield when it occurs at the vegetative stage, while the same deficit during the reproductive phase causes yield and spikelet number reduction by 20-70 % and 60 %, respectively. This can be accounted for by the phenotypic plasticity of cereals at early stages due to the trade-offs between yield components, i.e. panicle density, grain number per panicle and grain weight (Wey et al. 1998; Siband et al. 1999), particularly in rice where tillering is more effective than in maize or wheat.

Upland rice in Brazil is cultivated in large areas in the central Cerrado, where it is regularly subjected to drought but at distinct growth periods and with different intensities. Heinemann et al. (2015) classified upland ecosystems in three target populations of environment (TPE): one highly favorable environment (HFE) mainly stress-free, one favorable environment (FE) where mild water deficit occurs equally at the reproductive and terminal phases, and one least favorable environment (LFE) dominated by water deficit

74 occurring at the reproductive phase. They have concluded on the priority to improve
75 tolerance of upland rice to water deficit at the reproductive phase.

76 The high complexity of combining adaptive with productive traits in the same
77 genetic materials lowed the progress for improving genetic tolerance to drought (Kumar et
78 al. 2008). Breeding programs for tolerance opened two complementary ways: 1) the direct
79 selection on grain yield under drought, assuming that yield is the target objective beyond the
80 sequential mechanisms that generated it, focusing on the detection of QTL for yield and
81 yield components (Lanceras et al. 2004;Kumar et al. 2008; Serraj et al. 2011; Kumar et al.
82 2014, Kumar et al. 2018; Sandhu et al. 2019;); 2) the selection based on secondary traits,
83 that are defined as stable traits correlated to high yielding genetic materials in favorable
84 conditions and in predominant stress situations (Lafitte et al. 2003), relying on indicators of
85 tolerance (Subashri et al. 2009; Sellamuthu et al. 2011; He and Serraj 2012).

86 The present paper targets to identify, through fine phenotyping, the processes
87 triggered underwater deficit imposed during the reproductive phase and to assess which
88 elementary processes could be identified as adaptive, contributing to the maintenance of
89 yield and yield components under stress.

90 The reproductive phase starts with panicle initiation (PI) on the main tiller, a
91 period when plant faces the concomitance of vegetative organ growth and reproductive
92 structure formation (Counce et al. 2000, Figure 1). At plant level, tillering goes on after PI
93 and young tillers act as a sink for carbon, before becoming autotrophic (Matsuo and
94 Hoshikawa, 1993). At tiller level, demand in assimilate originates from the last four inner
95 developing leaves and the subsequent elongation of upper internodes and peduncle.
96 Complete expansion of these organs is critical as: 1) the three last leaves are responsible for
97 up to 86 % of carbon assimilate importation of the panicle in good conditions (Cock and
98 Yoshida, 1972) and 2) the stem and peduncle elongation drives the achievement of panicle

exertion and flowering ability. Any reduction in leaf expansion will thus reduce assimilate supply for stem elongation and panicle formation. Oppositely, if the source is performant, assimilate surplus can also be stored in stems and sheaths, in order to support efficiently grain filling after flowering, especially under drought conditions (de Raïssac, 1992). The period between PI and flowering is thus a time of high competition for carbohydrates within a tiller, between growth of existing vegetative organs and initiation/expansion of new reproductive structures, driving (i) the dimensioning of the future carbon source (elongating leaves); (ii) the panicle exertion ability (elongating internodes and peduncle); and (iii) the dimensioning of the ultimate carbon sink (branches and spikelets under development).

Figure 1 - Timing of developmental processes during the reproductive phase in rice

The occurrence of water deficit during the reproductive phase modifies this development pattern. Tillering decreases or -if maintained- generates mainly unfertile tillers (Alou et al. 2018), and plant growth rate is slowed down, which reduces grain number and potential grain size (Lilley and Fukai 1994a). In addition, flowering is systematically delayed (Boonjung and Fukai 1996b; Lilley and Fukai 1994a), up to 2 to 3 weeks depending on the intensity of the stress (Fischer and Fukai, 2003; Lafitte et al. 2003). Garrity and O'Toole (1994), and Swamy et al. (2017), found a negative correlation between the delay in flowering and yield, and proposed to use the delay as an indicator of plant sensitivity to drought (Subashri et al. 2009; Swamy et al. 2017). In some cases, the inflorescence does not emerge, partially or fully (Lafitte et al. 2003), preventing flowering. This delay and the poor panicle exertion can be associated with the lengthening in phyllochron and be the direct consequence of the decrease in peduncle elongation (Serraj et al. 2009; He and Serraj 2012), that accounts for 70-75% of spikelet sterility (O' Toole and Namuco, 1983). The reduction in branch and spikelet number, main components determining the potential yield, is another major effect of the reproductive water deficit (Lilley and Fukai 1994b; Boonjung and Fukai 1996a).

When the deficit occurs just before heading, it also affects pollen viability and the associated increase in spikelet sterility (Fischer and Fukai, 2003).

In conditions of limited CO_2 assimilation due to water deficit during the reproductive phase, the question raises in what extent (i) carbon is limiting for plant growth and development in terms of elongation processes and (ii) carbon investment into growing vegetative structures (leaves and stems) benefits or jeopardizes development of the reproductive structures (panicles). By comparing maize, soybean and sunflower, Boyer (1970) demonstrated that leaf enlargement decreases prior to photosynthesis in response to the reduction in water potential. This leads to an accumulation of sugars in plant organs, as observed in sunflower (Dosio et al. 2011), in coffee tree (Franck et al. 2006) or in rice (Luquet et al. 2006; Luquet et al. 2008; Rebolledo et al. 2012), giving evidence that growth is sink, rather than source, limited (Muller et al. 2011; Tardieu et al. 2018). Pantin et al. (2011) and Pantin et al. (2012) even proposed a switch from a metabolic to a hydro-mechanical limitation of leaf growth during the course of leaf ontogeny.

The present study analyses the response to the same reproductive water deficit of a set of six genotypes grown under two contrasted environments differing by the levels of incident radiation, air vapor pressure deficit and maximum temperature. It aims at exploring whether (i) the same mechanisms trigger the response to reproductive water deficit whatever plant growing conditions, (ii) there is synergy or competition between vegetative organ elongation and reproductive organ setting and development, and (iii) there are specific adaptive mechanisms and more tolerant genotypes able to preserve productivity during a reproductive water deficit.

Methods

Genetic materials

Six genotypes were used within two experiments. They were extracted from the PRAY japonica collection, retrievable on line (GRiSP- Global Rice Phenotyping Network, 2020). The selection was made in order to compare short cycle materials with a large genetic diversity and distinct geographic origins (Table 1). Data collected in previous experiments in Brazil and in controlled environments in France (Table 2) show that the genotype classification for cycle duration is only partially maintained between experiments, with an approximatively two week lengthening of duration in France.

Table 1.

Table 1 – Upland rice genotypes used in the trials Sitis and Growth Chamber.

Genotypes	Parents	Country of origin
CIRAD 392	Latsidahy x FOFIFA 62	Madagascar
CIRAD 409	CT11537 x CT10035	Colombia
EM IAC 165	Douradoprecoce x IAC 1246	Brazil
GUARANI	IAC 25 x 63-83	Brazil
HD 1-4	IRAT 146 x Beira Campo	France
IAC 25	Douradoprecoce x IAC 1246	Brazil

Table 2.

Table 2 – Duration in days from planting obtained from previous experiment.

Genotypes	Days to Flowering		
	Field 2014, Brazil	Growth Chamber 2016, France	Greenhouse 2008, France
CIRAD 392	58	75	71
CIRAD 409	56	69	70
EM IAC 165	61	79	71
GUARANI	58	71	77
HD 1-4	59	68	77
IAC 25	59	73	71

Sitis experiment (Sitis)

This experiment was conducted in Goiânia, Brazil, at Embrapa Rice and Beans, at an altitude of 823 m, latitude 16º28'00"S and longitude 49º17'00"W. The SITIS (Integrated System for Induced Treatment of Drought) phenotyping platform was used. The platform, set up in the greenhouse, is composed of a set of 100 cm high and 25 cm diameter PVC pipes, each one filled with soil and placed upon an automatic scale that allows a continuous

weighting of the pipes and monitoring of irrigation. The soil was ared latosol with medium texture, previously homogenized with a 1.25 cm mesh sieve to remove larger aggregates. The six genotypes were placed in a complete randomized block design with three replications and two water treatments: fully irrigated (IRR) and water stressed (STR) treatments, totalizing 36 plots. To secure three plants in each pot, a germination test has been carried out and plants were sown in excess on September 24, 2015. An application of 4 g of fertilizer NPK 04-14-08 was performed at planting, according to the recommendations for rice cultivation in relation with soil mineral analysis. Ten days after plant emergence, thinning was done to obtain three plants per pot. Climate conditions were monitored by an AKSO® device placed in the center of the greenhouse, continuously measuring air temperature and humidity, as well as solar radiation (Table 3).

Table 3.

Table 3 – Climate conditions in Sitis experiment.

	Min	Average	Max
T min (°C)	17.4	20.3	24.8
T max (°C)	24.6	31.8	37.6
Rad (MJ/m²/day)	1.9	16.7	26.0
VDP (kPA)	0.67	3.35	5.87

T min: minimal temperature; T max: maximal temperature; Rad: daily cumulated incident radiation; VPD: Air Vapor Pressure Deficit; Min and Max are respectively the minimal and maximal values monitored during the experiment; Average is the mean value during the same period.

Soil physical analysis provided values of 24.5% and 11.5% for field capacity (FC) and wilting point (WP) respectively. Before planting, pots were slightly over-irrigated. The water in excess drained off and the weight at field capacity was determined 24 hours after this irrigation. Taking into account PVC pipe and plate weights, soil weights at FC and WP were then calculated. To quantify water availability within the pots, the FTSW indicator, Fraction of Transpirable Soil Water, was used (Sinclair and Ludlow 1986). It was calculated as the ratio between the actual available water and the maximum available water, according to the following formula:

191 $$FTSW = \frac{(Actual\ soil\ weight - Soil\ weight\ at\ WP)}{(Soil\ weight\ at\ FC - Soil\ weight\ at\ WP)}$$

192 The pots of both treatments were adjusted daily to field capacity during the
193 whole vegetative phase and the beginning of the reproductive phase. Two days before water
194 stress application in the STR treatment, all pots were adjusted to FTSW = 0.8. The water
195 stress was applied in an "a priori" mode: using field data from Brazilian 2014 experiment
196 (Table 2), we applied water stress 20 days before the estimated flowering date, expecting a
197 duration of 10-14 days from PI. Stress application started on October 30, 2015 for Cirad 392,
198 Cirad 409 and Guarani, and on November 03, 2015 for EM IAC 165, HD 1-4 and IAC 25,
199 according to available data. After a 4-5 day period of dry-down, pots were daily adjusted to
200 FTSW 0.4 with a bottom-up irrigation: water was simply provided in the plate below the
201 pot, considering that observation confirmed that few primary roots had appeared at the base
202 of the pot for each genotype, meaning that the whole soil profile was colonized. Water deficit
203 treatment ended when at least 50% of main-tiller panicles of the irrigated treatment has
204 emerged (when at least 5 plants on the 9 plants in the 3 irrigated replications were at heading
205 stage).

206 In each pot, one plant tagged with a wool yarn and noted "plant 1" was observed
207 for non-destructive phenological traits up to the end of the differential water treatments. This
208 plant was then dissected while the two other plants of the pot were further grown at full
209 irrigation from heading to grain maturity and harvesting (results not presented here).

210 **Growth Chamber experiments (GC)**

211 Two experiments were carried out under the same climate conditions in a growth
212 chamber in Montpellier in 2016 and 2017: it was set up as a 12/12 hours photoperiod, with
213 day and night temperature and air humidity regulated at 28°C and 20°C, and at 65% and
214 90%, respectively (Table 4).

Table 4.

Table 4 – Climate conditions in Growth Chamber experiment.

	Min	Average	Max
T min (°C)	19.9	20.1	21.6
T max (°C)	26.7	27.8	28.4
Rad ($MJ.m^{-2}.day^{-1}$)	1.3	5.8	9.0
VDP (kPA)	1.26	1.41	1.54

T min: minimal temperature; T max: maximal temperature; Rad: daily cumulated incident radiation; VPD: Air Vapor Pressure Deficit; Min and Max are respectively the minimal and maximal values monitored during the experiment; Average is the mean value during the same period.

A first methodological experiment addressed the choice of soil substrate and the intensity of water deficit, as well as checking genotype cycle duration from planting to flowering. It was a complete randomized design with six genotypes and one water treatment (full irrigation). After germination, planting was achieved on 20 September, 2016. The number of days to panicle initiation was estimated by using the Haun index method (see below). Duration from planting to panicle initiation varied between genotypes, from 26.4 days with HD 1-4 to 36.3 days with EM IAC 165. The reference genotype IR 64 was added, to which two levels of water deficit were applied from 10 days after panicle initiation to panicle emergence: one adjusted each two days to FTSW 0.4, the other to FTSW 0.2, meaning that FTSW was lower than its ceiling value between two consecutive irrigations. The FTSW 0.2 treatment was finally too severe as it prevented any development of the plants during the reproductive phase. The FTSW 0.4 treatment was then selected (as a “mild” water deficit) for the final experiment.

The second and main experiment was conducted in a completely randomized design with six genotypes grown under two water treatments, irrigated (IRR) and stressed (STR), in three replications for a total of 36 pots. Each replicate was composed of a 3.5 liter polyethylene pot containing one single plant. Seeds were pre-germinated in an incubator set up at 30ºC for three days. Three seedlings were transplanted on 5 January, 2017 and subsequently thinned to one plant per pot. The pots were filled with 1470 grams of a

commercial referent substrate (Tref Riz Cirad 2), specifically adapted to rice, mixed with seven grams of Basacote 6M+, a slow-release fertilizer. For each pot, a soil sample was weighted and oven-dried in order to determine the initial effective soil humidity and the real soil dry weight. Pot weights at FC and at WP were then deduced in order to calculate FTSW. The water deficit treatment was applied as in the Sitis experiment on an "a priori" mode: water irrigation was with hold ten days after the date of panicle initiation estimated from the pre-experiment. At the beginning of the water deficit application, the pots were covered with polystyrene micro balls to avoid water loss by soil evaporation, and to measure the plant water consumption between two consecutive irrigations. The exact quantity of water was then supplied every two days by top irrigation in order to adjust to FTSW 0.8 and 0.4 in IRR and STR treatments, respectively. The genotype-based water deficit treatment was maintained up to panicle emergence on the main tiller of the irrigated plants. Plants were then dissected and dried to obtain the partitioning of plant biomass.

Measurements in both experiments

Phenological development monitoring

Mean air temperature was calculated on a daily step in both experiments. Thermal time was calculated using Samara model values (Kumar et al. 2017), with following cardinal temperatures: Tb = 10, Topt1 = 28, Topt2 = 36, Tmax = 44.

The Haun Index (HI, Haun, 1973), which is calculated as the number of leaves on the main stem whose ligules is visible plus the ratio to full elongation of the portion of the visible blade length of the youngest leaf, was monitored three times a week on the main tiller of each plant. The phyllochron was calculated as the time -in days or thermal time- separating the ligules emergence of two successive leaves of the main tiller. The early phyllochron was determined before PI and under non-limiting water supply for all genotypes, so between 14 and 27 days after planting (DAP) in Sitis, and between 11 and 24

DAP in GC. Phyllochron was also determined between the onset of water treatments and ligules emergence of the flag leaf (Phyllochron PI to Max HI).

The date of PI was retrospectively estimated for each plant, according to the method illustrated in Figure 2 for Cirad 409 grown in GC under irrigated conditions: because the final leaf number (FLNb) on the main tiller is obtained at panicle emergence and because PI is known to occur at FLNb-4 (Nemotoet al. 1995), the precise date of PI is estimated by interpolation between the two consecutive phenological observations comprising the target PI. In the illustrated case (Figure1), FLNb is 10, PI is consequently estimated at 6HI and PI date estimated by interpolation at 34 DAP. This method was applied to all individual plants in both experiments. Plant height, defined as the distance between the soil and the last ligules on the main stem, was measured twice a week. Panicle emergence stage was determined when more than 50% of main tiller panicles of one genotype has emerged within the irrigated treatment.

Figure 2 - Determination of the panicle initiation date with Cirad 409 under irrigated condition in the growth chamber experiment

At panicle emergence in both experiments, after tiller counting, plant dissection has been done, to determine the following data:

- main-tiller internode 1 and 2 length and width (diameter), considering internode 1 as the first internode below the peduncle;
- main-tiller panicle, leaf and node dry weight;
- main-tiller first and second order branches number;
- main-tiller spikelet number;
- remaining tiller dry weight;

In addition, the length and width of all leaves of the main tiller were measured as soon as they were fully emerged. Specifically in Sitis experiment, plant dry weight was

289 measured at thinning at 14 DAP. Specifically in GC experiment, the panicle main axis length
290 and the total length of branches primary and secondary were measured.

291 **Carbohydrate analysis**

292 Flag leaf blade (FL) and internodes 1 and 2 (IN1 and IN2) were sampled early
293 morning, or the beginning of the light period and plunged into liquid nitrogen right after
294 sampling, freeze-dried (72h), grinded with a ball mill Retsch MM400 (particules < 50 µm)
295 and then stored in a freezer at -80°C. Sugar content was measured in Sitis according to the
296 method described by Gibon et al. (2009) and in the growth chamber by Luquet et al. (2006).
297 The sugar extractions and starch hydrolysis are similar with two methods, the
298 methodological difference concerns only the sugar quantification. The first method uses
299 spectrophotometry and the other one is based on high performance liquid chromatography.
300 Samples were previously used to ensure that the results could be comparable. The results are
301 expressed as glucose equivalents per unit dry matter ($mg.g^{-1}$) for starch (Starch) and as
302 soluble sugar (SolSug) per unit dry matter ($mg.g^{-1}$) corresponding to the amount in hexoses
303 and sucrose.

304 **Gas exchange measurement**

305 In Sitis, transpiration was measured at the end of the experiment, between 9 and
306 11 a.m., with the flag leaf of each plant n°1, using the LC-Pro-IRGA® device, regulated at
307 a 300 $ml.min^{-1}$ air flow with a 1200 $\mu mol.m^{-2}.s^{-1}$light source.

308 In the growth chamber, photosynthesis and transpiration were measured the day
309 before dissection by the Walz GFS 3000.

310 **Derived variable calculation**

311 The main tiller elongation rate was calculated as the increase in height during
312 the stress period (in IRR and STR plants) divided by the number of days. An estimation of
313 the relative plant growth rate (RGR) was calculated differently in Sitis and GC experiments.

314 In Sitis, genotype shoot dry weight was measured on six plants from the IRR treatment at 14
315 DAP-at time of thinning- and at panicle emergence. RGR was then calculated for IRR
316 treatment as following:

317 $$RGR = \frac{\Delta \text{ Ln (shoot dry weight)}}{\Delta \text{Date}}$$

318 The mean genotype RGR was then used to estimate by intrapolation shoot dry
319 weight of plants at the onset of water deficit application. Considering shoot dry weight at the
320 onset of water deficit and at heading (end of the experiment), RGR ($g.day^{-1}$) and GR (Growth
321 rate - $g.day^{-1}$) were calculated during the whole period when differential water treatments
322 were effective. In the growth chamber, an allometric correlation between height and biomass
323 was used to estimate the shoot dry weight of the plants at the onset of water deficit
324 application.

325 In GC, because pots were covered by polystyrene bills preventing direct soil
326 evaporation, the monitoring of pot weights before and after irrigation allows calculating the
327 daily plant transpiration, as well as a cumulated plant transpiration during the differential
328 water treatment period. The cumulated water use efficiency was then calculated as the ratio
329 of the increase in biomass to the amount of water consumed during the same period.

330 To quantify genotype response to water deficit, response indexes were calculated
331 as the relative variation between IRR and STR treatments, as defined below:

332 $$Responde\ index = \frac{(STR - IRR)}{IRR}$$

333 Where:

334 STR is the genotype adjusted mean of the considered variable for the water deficit treatment;
335 IRR is the adjusted mean of the considered variable for the irrigated treatment.

336 **Statistical analysis**

337 Anovas were run using R packages “agricolae” (de Mendiburu 2014),
338 “ScottKnot” (Jelihovschi et al. 2014), “doBy” (Hojsgaard et al. 2019) and “ggplot2”
339 (Wickham 2016). The response indexes were validated by a test of orthogonal contrasts,
340 where the adjusted means of the IRR treatment were compared to the adjusted means of the
341 STR treatment, using F Test and R software.

342 **Results**

343 **Impact of the timing of water deficit on developmental processes.**

344 Identification of the precise dates of occurrence of water deficit for each
345 genotype was determined before going through any analysis. First, the date of panicle
346 initiation (PI) was retrospectively estimated for each individual plant, as described in
347 Methods. The time lag between PI and the actual start of water deficit application was then
348 determined: a global index of the relative progress of the reproductive phase at time of stress
349 application was calculated, as the ratio (in days) of the elapsed time from PI to the onset of
350 water deficit to the total reproductive phase duration (Table 5).

351 **Table 5.**

352 **Table 5 – Mean progress of reproductive phase at start of water deficit application.**

Genotypes	Trial	Heading (DAP)	Water deficit application (Days after PI)	Reproductive phase duration (Days)	Reproductive phase achieved (%)
CIRAD 392	**SITIS**	53	15.0	32.0	46.8
CIRAD 409		47	15.3	26.3	58.1
EM IAC 165		54	16.0	30.0	53.3
GUARANI		53	13.0	30.0	43.3
HD 1-4		54	17.7	31.7	55.8
IAC 25		54	16.0	30.0	53.3
Mean		**52.5**	**15.6**	**30.1**	**51.8**
CIRAD 392	**Growth Chamber (GC)**	68	7.0	41.0	17,0
CIRAD 409		59	2.0	31.0	6,4
EM IAC 165		71	9.0	45.0	20,0
GUARANI		64	3.0	34.0	8,8
HD 1-4		62	5.0	36.0	13,9
IAC 25		64	10.0	39.0	25,6
Mean		**64.6**	**6.0**	**37.7**	**15.3**

353

354 The application of water deficit occurred earlier in GC than in Sitis: the average
355 relative progress of the reproductive phase was 15.3 % in GC, and 51.8 % in Sitis. This was
356 mainly due to a lack of reliable field data in Sitis to predict plant cycle duration and so to
357 estimate time of PI, while the pre-experiment in GC with controlled conditions helped to
358 overcome this difficulty. Thus, the water deficit was applied later in Sitis than in GC, but
359 with the same intensity as determined by pot water content adjusted at FTSW 0.4 each two
360 days on both experiments.

361 The rate in time to which each single phenological process was affected due to
362 the application of water deficit was finally computed for each genotype and in each trial
363 (Tables 6 and 7), with reference to the timing and position of each one of these processes
364 (Figure 1). To develop a subsequent reliable analysis, these rates were used as the basis to
365 only study the processes that were completed during water deficit.

366 **Table 6.**

367 **Table 6 – Impact of water deficit on reproductive phase processes in Sitis.**

368 **Table 7.**

369 **Table 7 – Impact of water deficit on reproductive phase processes in Growth chamber.**

370 One key element of this study was to quantify the effect of water deficit on any
371 phenological processes that occurred at the early reproductive phase (flag leaf formation,
372 bract formation) since these processes were over before the end of the water treatment which
373 occurred at panicle exertion in the irrigated plants. So, in the water stress treatment, these
374 processes were fully affected by water deficit in one or both experiments. This was, however,
375 irrelevant to quantify the effect of water deficit on processes that were still on-going heading,
376 like internode 1 and peduncle elongation, or pollen viability. Indeed, at the time of high
377 elongation rate of internode 1 or peduncle, any slight delay in development due to the
378 application of water deficit can lead to huge differences in internode and peduncle lengths,

as measured with destructive observation. Any differences between treatments were thus the consequence of the effect of water deficit but also of the likely delay in plant development. In this way, the inter-genotype and inter-treatment variability in peduncle length data were huge – and in a lower extent that in internode 1-, but that has included a strong effect of the delay in phenology. These variables, as well as pollen viability, have not been considered for further analysis

The robustness of the estimates at Tables 6 and 7 was confirmed by the comparison of the flag leaf length. The water deficit was not applied during the leaf elongation phase in Sitis while it was in GC, hence, no effect was expected in Sitis to the difference of GC. An anova conducted separately on both experiments confirmed this hypothesis, with a significant effect in GC only (Table 8 and Figure 3). As no Treatments X Genotypes interaction was observed in GC, it is reported that leaf elongation across genotypes reacted the same way to water availability.

Table 8.

Table 8– Leaf length analysis of variance in Sitis and Growth Chamber.

	Sitis			Growth Chamber		
SV	**DF**	**Pr (>F)**	**Significance**	**DF**	**Pr (>F)**	**Significance**
Block	2	0.86	NS	-	-	-
Treatments	1	0.40	NS	1	**0.00**	***
Genotypes	5	0.27	NS	5	0.30	NS
Trmnt. X Genotypes	5	0.22	NS	5	0.96	NS
CV%		11.79			19.36	

Means were calculated on 6 genotypes x 3 replicates (18 values) for each water treatment in each experiment. The response index was calculated as described in Material & Methods. NS: Non significant; ***: significant at $P < 0.001$

Figure 3 - Flag leaf length in relation with the experiments and water treatments.

Lower internode elongation was not submitted to water deficit in Sitis, while it was partially in GC. As reported earlier, some other processes were fully affected since their initiation (so reported as 100% in Tables 6 and 7) but were likely interrupted before their completion (Internode 1 and peduncle elongation, pollen viability) due to the end of the

experiment. These processes were excluded from the analysis. In contrast, some processes were affected by water deficit, partially in Sitis and totally in GC (see Tables 6 and 7), like branching I and II (32 and 100% respectively), internode 2 elongation (36 and 100% respectively) and spikelet formation (88 and 100% respectively). These were completed at the end of experiment and are the main processes that will be investigated in the subsequent analyses of this paper.

Effect of environmental conditions on plant development rate and cycle duration

The cumulated thermal time from planting to heading increased quicker in Sitis than in GC (Figure 4). The slope, indicating the mean daily cumulated thermal time, was 17.5 °C days and 13.7 °C days respectively. The slower accumulation of thermal time from planting to heading in GC accounted for its longer growth period duration (in days), the flowering date occurring 16.7 days later and the reproductive phase lasting 6.5 days longer (37.7 vs. 30.1 days, Table 5) in GC than Sitis. Based on thermal time, the early phyllochron was also longer in GC than Sitis for the six genotypes. The ratio of phyllochron of GC to Sitis ranged from 1.36 in Cirad 409 to 1.17 in IAC 25. Another cause of the rise in cycle duration in GC was the increase in the final leaf number on the main tiller (Table 9), between 1 to 1.5 leaf respective of the genotype. Conclusively, the longer cycle duration (in days) in GC is due to the combined effect of a lower thermal time accumulation, a lengthening in phyllochron measured in ^{o}days and a higher number of emitted leaves.

Figure 4 - Cumulated thermal time in Sitis and Growth Chamber experiments in function of calendar time

Table 9.

Table 9 – Final leaf number on the main stem in Sitis and Growth Chamber experiments.

Genotypes	SITIS	Growth Chamber
CIRAD 392	9.0	11.3
CIRAD 409	8.7	10.3

EM IAC 165	9.0	10.2
GUARANI	9.2	11.2
HD 1-4	8.7	9.5
IAC 25	9.0	10.0

426

427 **Effect of environmental conditions on plant morphological and physiological traits and**
428 **their response to water deficit**

429 Relevant traits that were affected by water deficit in at least one experiment and
430 whose processes were completed at heading were studied in a split-plot analysis. This
431 analysis considered that both experiments display a completely randomized design, with the
432 first factor (the experiments) at two levels and the second factor (water treatments) at two
433 levels, the genotype factor not being considered. Data are shown at Table 10.

434 **Table 10.**

435 **Table 10 – Effect of experiment conditions and water treatments on rice plant traits.**

	Means				Response Index		Significancy		
	Sitis		Growth Chamber		Sitis	Growth Chamber	Exp	Trtmnt	Exp x Trmnt
Traits	Irrigated	Stressed	Irrigated	Stressed					
TillNb	9.7	8.7	6.8	6.9	-0.11	0.01	***	NS	NS
Plant Height (mm)	697.9	561.4	813.4	689.2	-0.20	-0.15	***	***	NS
Plant Biomass (g)	16.2	13.0	15.7	13.2	-0.20	-0.16	NS	***	NS
GrowthRate (g.day^{-1})	1.14	0.90	0.40	0.33	-0.21	-0.18	***	***	NS
FLLength (mm)	436.5	428.1	604.2	499.3	-0.02	-0.17	***	***	***
FLWidth (mm)	17.3	17.3	18.7	17.3	0.00	-0.07	NS	NS	NS
IN2Length (mm)	141.3	104.1	140.3	119.3	-0.26	-0.15	NS	***	NS
IN2Width (mm)	5.6	5.3	5.9	5.1	-0.04	-0.13	NS	***	NS
TotalBranchNb	35.2	29.8	37.9	28.2	-0.15	-0.26	NS	***	NS
SpikeletNb	155.2	131.9	156.7	118.8	-0.15	-0.24	NS	***	NS
ElongRate (mm.day^{-1})	26.3	16.3	14.6	11.1	-0.38	-0.24	***	***	***
FLSolSug (mg.g^{-1})	66.0	108.7	60.7	72.9	0.65	0.20	***	***	***
FLStarch (mg.g^{-1})	54.5	47.5	0.7	0.8	-0.13	0.14	***	***	***
IN2SolSug (mg.g^{-1})	55.2	104.9	78.4	88.7	0.90	0.13	NS	*	NS
IN2Starch (mg.g-1)	128.4	108.1	25.8	24.5	-0.16	-0.05	*	NS	NS
LeafTransp (mmol.m^{-2}.s^{-1})	4.6	3.9	4.4	3.8	-0.15	-0.14	NS	*	NS
Phyllochron (days)	7.9	9.5	7.8	8.5	0.21	0.09	NS	NS	NS

436 Means were calculated on 6 genotypes x 3 replications (18 values) for each water treatment in each experiment.
437 The response index was calculated as described in Material & Methods. NS: Non significant; *: significant at
438 $P < 0.05$; **: significant at $P < 0.01$; and ***: significant at $P < 0.001$.
439

Under irrigated conditions, whole plant growth rate during reproductive phase decreased by 64 % from Sitis to GC (from 1.14 to 0.40 $g.day^{-1}$) which was associated with a 65 % decrease in incoming radiation (from 16.7 $MJ.m^{-2}.day^{-1}$ to 5.8 $MJ.m^{-2}.day^{-1}$), and a 24 % decrease in maximum daily temperature (from 37.6 to 28.4 °C, see Tables 3 and 4), leading to a significant reduction in tiller number per plant (6.8 in GC vs. 9.7 in Sitis). In the same line, while concentration of soluble sugars was in the same range across both environments in the flag leaf (66.0 $mg.g^{-1}$ in Sitis vs 60.7 $mg.g^{-1}$ in GC) and in internode 2 (55.2 $mg.g^{-1}$ in Sitis vs 78.4 $mg.g^{-1}$ in GC), that of starch was drastically reduced from Sitis to GC for these 2 organs (from 54.5 $mg.g^{-1}$ to 0.7 $mg.g^{-1}$ in flag leaf and from 128.4 $mg.g^{-1}$ to 25.4 $mg.g^{-1}$ in internode 2). Interestingly, as a consequence of the delay in heading time in GC, the final whole plant biomass was not significantly different between both experiments (16.2 g in Sitis and 15.7 g in GC). The extended growth duration in GC contributed in generating taller plants than in Sitis (813.4 mm vs. 697.9 mm) whereas stem elongation rate was significantly lower(14.6 $mm.day^{-1}$in GC vs 26.3 $mm.day^{-1}$ in Sitis). Similarly, flag leaf was longer in GC (604.2 mm) than in Sitis (436.5 mm). These results are in accordance with the reduction of leaf elongation observed in maize in response to high VPD, as it was in S (Tardieu 2005; Bouchabké et al. 2006). Like plant biomass, some other traits relative to organ morphology and number determined during the reproductive phase were not significantly modified across growing conditions: no significant differences were observed for internode 2 length (141.3 mm vs 140.3 mm), internode 2 width (5.6 mm vs 5.9 mm), total branch number (35.2 vs 37.9) and spikelet number (155.2 vs 156.7). The transpiration rate of the flag leaf of the main tiller measured right before heading did not also differ between experiments.

Experiment x Treatment interactions were revealed for some traits (Table 10) and can be explained by two main reasons. The first one is the lag in the timing of water

deficit applications between both experiments: respective to the physiological time, the deficit was applied later in Sitis than in GC and so did not affect some processes the same way. As already seen, flag leaf length was not affected in Sitis, but was in GC as its elongation was already over when the deficit was applied in GC. Similarly but not significantly, flag leaf width was not affected in Sitis but was in GC. The second reason is directly related to environment conditions: in the flag leaf, in response to water deficit, soluble sugars increased poorly in GC (from 60.7 to 72.9 $mg.g^{-1}$) and strongly in Sitis (from 66.0 to 108.7 $mg.g^{-1}$) (Table 10). At the same time, starch content was stable and nearly negligible in GC (from 0.7 to 0.8 $mg.g^{-1}$) while it decreased in Sitis (from 54.5 to 47.5 $mg.g^{-1}$), in agreement with previous results (Rebolledo et al. 2012) and in accordance with the low radiation and the consecutive poor carbon availability in GC. No other interactions in response to water deficit has been detected with the other traits under study, i.e. plant height and biomass, internode 2 length and width, branch and spikelet number and final leaf transpiration. As an example, a strong correlation was reported for the length of internode 2 between values reported from GC and those reported from Sitis (Figure 5), giving consistency to our results.

Figure 5 - Relationship between internode 2 length in Growth Chamber and Sitis experiments

Genotype response to water deficit at reproductive phase

The integrated response of the six genotypes to water deficit was analyzed based on the response indexes as defined in Methods. The PCA on the response indexes of all the studied morphological and physiological traits was run using the variations of total branch number (ΔTotBranchNb) and spikelet number (ΔSpikeletNb) as supplementary variables: these two variables were considered as the final products of the plant at the stage when the

trial was stopped (heading) and so the best indicators of plant adaptability to water deficit established during the reproductive stage. The correlation matrix is given in Table 11.

Table 11 – Correlations matrix of the studied variables.

As expected, ΔTotBranchNb and ΔSpikeletNb were highly correlated (+0.945***) to each other. The ΔTotBranch Nb was highly correlated to ΔFLLength (+0.730**), ΔFLWidth (+0.736**) and ΔIN2Width (+0.728**): the reduction in flag leaf and internode 2 dimensions in response to water deficit was closely associated with the reduction in the panicle branch number. The ΔSpikeletNb was correlated also to the same variables at 5% (*). The ΔLeafTransp was also positively correlated to ΔSpikeletNb (+0.578*) and logically more weakly to ΔTotBranchNb (+0.526°), as the formation of branches occurs prior to spikelet formation.

In addition, ΔTillNb was the only trait negatively correlated to ΔTotBranchNb (at 10%), while it was positively and strongly correlated to ΔGrowthRate. And ΔGrowthRate did not display any correlation with other traits, representative neither of organ growth (morphogenesis), or of organ generation (organogenesis). The ΔElongRate was positively correlated to ΔIN2Length (**) and ΔFLStarch (*), and negatively to ΔPhyllochron (**) and ΔFLSolSug (*).The ΔPhyllochron was positively correlated to ΔIN2SolSug.

The PCA representation on the two first components explained 55.4% of the total variability (Figure 6). The first component (31.2%) was principally defined (i) positively by ΔElongRate and ΔIN2Length and (ii) negatively by ΔFLSolSug and ΔPhyllochoron. The second component (24.4%) was defined positively by ΔIN2Width and ΔFLWidth and negatively ΔTillNb.

Figure 6 - Principal Component Analysis representation on the two first components of the response index

513 The supplementary variables ΔTotBranchNb and ΔSpikeletNb were both almost
514 fully positioned positively on the second component, and tightly associated with the
515 maintenance of flag leaf and internode 2 diameters (ΔFLWidth and ΔIN2Width). Tiller
516 number variation was fully opposed to yield components and was the unique trait displaying
517 high negative correlations with them (-0.511 and -0.470 with ΔTotBranchNb and
518 ΔSpikeletNb, respectively): this correlation means that the more tillers were initiated under
519 water deficit, during the reproductive phase, the less branch and spikelet number was set in
520 the main tiller panicle.

521 A strong difference between Sitis and Growth Chamber experiments and a large
522 gradient of genotype responses are visible in Figure 6 and Table 12. In both experiments,
523 water deficit was associated with a reduction in the stem elongation process (ΔElongRate,
524 ΔIN2Length) and organ starch content (ΔFLStarch and ΔIN2Starch), while phyllochron and
525 soluble sugar content increased (ΔFLSolSug and ΔIN2SolSug), illustrated by the genotype
526 gradient along the PCA first component (Figure 6). In contrast, along the second component,
527 the genotype increase in soluble sugar was rather associated with the maintenance in yield
528 components in Sitis but not in GC. Interestingly, the singular behavior of Cirad 409,
529 minimizing its response to water deficit in both experiments, is noticeable.

530 **Table 12.**

531 **Table 12 – Response index in Sitis and in Growth Chamber experiments for all studied**
532 **traits and genotypes.**

533 In Sitis, no trait variation was observed with Cirad 409, except for flag leaf starch
534 (ΔFLStarch), which was reduced by 28%, while the reduction was 39% in IAC 25 and 13%
535 in EM IAC 165. As the most sensitive trait, soluble sugars increased up to 97% in the flag
536 leaf (HD 1-4) and to 148% in the internode 2 (Cirad 392). Main tiller elongation rate was
537 also very sensitive to water deficit, with significant variations ranging between 31% (HD 1-

4) and 55% (EM IAC 165). Variations of total branch number were smaller, mainly non-significant, except in IAC 25 and HD 1-4 (-31% and -23% respectively). Spikelet number was also poorly affected, except in IAC 25 (- 33%), confirming the weak adaptability of this genotype. Interestingly, values of yield components were maintained under water deficit with Cirad 392, while significant variations in stem elongation and soluble sugar contents were reported.

In the Growth Chamber, no significant response index was observed with Cirad 409: in this genotype, all variables were maintained unchanged under moderate water deficit, as observed in Sitis (with the exception of ΔFLStarch). The evolution is quite different with other genotypes. Variation in flag leaf soluble sugars was very poor compared to Sitis, only Guarani and EM IAC 165 displayed a significant increase by +27 % and +29%, respectively. Oppositely, variation in soluble sugars of internode 2was remarkably contrasted depending on the genotype: it ranged from a reduction of 62% with Cirad 392, with a stability with HD 1-4, to an increase of 191% with EM IAC 165. And the sensitivity of the elongation rate to water deficit was also remarkable within all genotypes except with Cirad 409: it decreased from 17% (Guarani) to 34% (Cirad 392). Finally, except for the case of HD 1-4, yield components were more affected in GC than in Sitis: EM IAC 165 and IAC 25 were the most sensitive genotypes, with reduction in branch number by 46 and 25 % and spikelet number by 45 and 26%, respectively.

Discussion

In this study, we have investigated the response to a water deficit applied during the reproductive phase of six rice genotypes grown under two contrasted climate conditions: high temperatures, high radiation and VPD in Sitis; optimum temperatures, low radiation and VPD in GC.

Phenotyping for tolerance to mild water deficit at reproductive stage

A varietal comparative analysis of the effect of a water deficit during the reproductive phase in cereals requires applying the constraint at the same phenological age for all varieties. Indeed, considering the precise timing of initiation and development of each organ during that phase, any time lag in the phenology of two varieties would prevent from comparing the effect of a constraint established at a single date on plant traits, like organ size or yield components (grain weight, grain and spikelet number). Indeed here, a strong correlation between time of water deficit application and its impact on organ development was highlighted: flag leaf length was not affected when water deficit application was delayed (Sitis), but was in case of an earlier application (Growth Chamber). One major challenge of the present study was yet to apply the water deficit at the same phenological age whatever the genotype. This was achieved in Montpellier due to: i) a preliminary dedicated trial whose goal was to observe the respective phenology of each variety; ii) a highly controlled and identical environment for both the preliminary and the main trials. However, this was only partially achieved in Sitis as the daily variation of radiation, temperature and photoperiod has impacted plant phenology and prevented the authors from (a) considering the preliminary available field data as useful to predict plant phenology and (b) setting up an initial trial (which should have been organized a year earlier) because of the photoperiod sensitivity.

The use of thermal time, instead of days, is widely accepted as a common way to overcome this difficulty to take into account phenological stage durations. Underlying the assertion that development rate is driven by temperature (Jamieson et al. 1995), thermal time turns possible the comparison of plant phenology across environments despite their differences in temperature (Bouman et al. 2001). However, the rate of leaf emergence in the present study, even expressed in thermal time, differed across trials, the phyllochron being shorter in Sitis than in GC. We hypothesized here that the temperature effect may have been

588 modified by an additional effect of daily radiation, the later one displaying average values
589 of 5.8 $MJ.m^{-2}.day^{-1}$ in GC and 16.7 $MJ.m^{-2}.day^{-1}$ in Sitis. In rice, Islam and Morison (1992)
590 introduced the photothermal quotient (PQ) - as the ratio between cumulated radiation and
591 cumulated thermal time ($MJ.m^{-2}.°C^{-1}$) - during the 30 days- day period before harvest in
592 order to predict yield. Recently, Baumont et al. (2019) indicated in wheat the joint role of
593 temperature and carbon availability on plant development rate and concluded that the use of
594 photothermal quotient (PQ) is relevant to better model cycle durations in different light and
595 temperature environments. In order to compare phyllochron between environments, it is
596 suggested here that thermal time is appropriate when environments differ mainly by their
597 temperature conditions, while PQ is when facing contrasted environments, differing by
598 incident radiation, temperature and VPD, as it was the case in our study.

599 The phenological framework described here in Figure 2 allowed to determine a
600 posteriori the PI dates for each genotype and to quantify how much in time each process of
601 plant development has been affected by the water deficit (Figure 1, Tables 6 and 7). This
602 was possible even without a tight control of the onset of water deficit in relation with the
603 timing of the reproductive phase. A comparative analysis between sites of the effect of water
604 deficit, having the same intensity and timing between genotypes within one experiment,
605 could then be run between the two sites, as long as it has focused on traits that were actually
606 affected by water deficit in both experiments.

607 This a posteriori determination of the PI date shall be particularly useful in field
608 conditions, within breeder's trials or large-scale phenotyping trials, where the triggering of a
609 water deficit cannot be driven at the plant or genotype level, as we did with plants in pipes
610 or in pots. Indeed, in case of a large plant diversity within the breeder's field, the occurrence
611 of a single water deficit period might affect plants at quite distinct stages (some might be
612 already in the reproductive while others are still in the vegetative). In the field, to deal with

613 a large panel of genotypes differing in earliness, and so to minimize this discrepancy in
614 phenology, several methods have been used in the literature. Garrity and O'Toole (1994)
615 synchronized the flowering dates of 55 cultivars by clustering genotypes and by planting
616 date respective of their cycle duration (based on previous data). In contrast, Lilley and Fukai
617 (1994) triggered water deficit at a single fixed time after PI had occurred for all materials.
618 In the same line, Subashri et al. (2009) used the same date for establishing the water deficit
619 accepting some slight differences in earliness within a set of near genetic materials. Another
620 time-consuming option implemented by Sellamuthu et al. (2011) is to regularly dissect extra
621 plants and withhold irrigation when 50% of the studied genetic population is at PI, but with
622 the high probability of not impacting the reproductive traits with the same intensity in all
623 materials. Practically, it is proposed here to control the genotype development upstream and
624 downstream by: (1) using PQ to better estimate the development rate of genotypes across a
625 large range of environments, (2) conducting an initial trial for flowering time observation
626 and clustering genotypes with respect to their crop duration, (3) running the main trial with
627 distinct sowing dates respective of genotype clusters, (4) monitoring Haun Index all along
628 the experiment in order to identify a posteriori the actual dates of PI and the genotype traits
629 effectively affected by water deficit, that can be statistically compared for water deficit
630 tolerance.

631 **Stability of water deficit response through contrasted climate conditions**

632 Comparing plant growth in favorable water conditions between the two sites was
633 the first step to overcome before analyzing the response of genotypes to water deficit. The
634 sites were discriminated respective of temperatures and VPD which were lower in GC
635 compared to Sitis, and of incoming radiation which was even reduced by 63% in GC. This
636 led to a drastic reduction in plant growth rate, elongation rate and leaf appearance rate, in
637 GC compared to Sitis. In compensation, cycle duration -in days and in thermal time- was

longer in GC than in Sitis. Finally, no significant differences in plant biomass or plant height between sites were observed, highlighting the existence of trade-offs between phenological rates and duration. Thus, Cookson et al. (2005) observed in leaf of *Arabidopsis thaliana* that initial expansion rate was negatively correlated to the duration of expansion. Using radiation treatments, Chenu et al. (2005) also found an increase in duration along with a decrease in elongation rate under low radiation treatment, while Cookson and Granier (2006) observed that the trade-off was not complete. Oppositely, when *Arabidopsis thaliana* was submitted to water deficit, Aguirrezabal et al. (2006) found a complete compensation between the extended duration and the decrease in elongation rate, supporting our own results in rice when submitted to water deficit.

The contrasted climate conditions associated with the same intensity in water deficit provided here the appropriate framework to evaluate whether or not any detected relationships characterizing plant response to water deficit are strong enough to be valid in a large range of environments. In our study, considering that the trial in GC ended at heading, we considered the maintenance of the reproductive sink size (branch and spikelet number) as the best indicator of water deficit tolerance, since it guaranteed yield potential. By combining the two trials, the analysis showed that the variation of spikelet and branch number was apparently independent of the variation in elongation rate (Table 11), meaning that the maintenance of organ elongation cannot be related to the maintenance of development of the reproductive organs. Nevertheless, at trial level, the global trend was not validated and environment specific plant responses to water deficit were reported: the more a genotype reduced internode elongation and increased soluble sugar content under water deficit, the more sink size (spikelet and branch number) was maintained in Sitis but reduced in GC (Figure 6). This observation is likely related to the difference in plant carbon availability. This emphasizes how much the response of these traits to water deficit is

663 dependent on climate conditions, and how conclusions often have to be restricted to the
664 conditions under study.

665 Other correlations are conserved across the two conditions. This is the case with
666 the reduction in elongation rate, highly associated here with an increase in soluble sugars:
667 soluble sugar content in plant organs is widely reported as increasing in response to water
668 deficit, because organ elongation is reduced before any effect on leaf photosynthesis is
669 detected (Boyer 1970; Muller et al. 2011). Thus, the plant diminishing carbon demand leads
670 to an accumulation of sugars in source leaf and sink organs, which in turn appears as a good
671 indicator of stress perception. Equally, the decrease in elongation rate is highly correlated to
672 phyllochron lengthening, generating a delay in panicle emergence, trait widely considered
673 as indicative of plant sensitivity to water deficit (Garrity and O'Toole 1994; Boonjung and
674 Fukai 1996b; Subashri et al. 2009; Sellamuthu et al. 2011; He and Serraj 2012; Sheoran et
675 al. 2014; Swamy et al. 2017). This is confirmed in GC but not in Sitis. We hypothesize high
676 VPD and radiation in Sitis –and the low radiation and VPD in GC- are responsible for the
677 differential responses to water deficit across trials.

678 **Tolerance criteria and genotype performance**

679 Flag leaf width and internode 2 diameter reduction were tightly correlated to sink
680 size (Total Branch Number and Spikelet Number) reduction in both trials, whereas most of
681 the other traits under investigation were not. This could be associated with the size of the
682 apical meristem. Matsushima (1966) form early highlighted a strong correlation between
683 internode 1 thickness and spikelet number of the panicle. Likewise, Dingkuhn et al. (2015)
684 and Adriani et al. (2016) observed a high positive correlation between flag leaf area and
685 spikelet number of the panicle. Kobayasi et al. (2002) demonstrated with different nitrogen
686 treatments the correlation between the apical dome diameter at panicle initiation and the
687 number of branches and spikelets of the panicle. And comparing wild (*Oryza Barthii*) and

688 domesticated (*Oryza Glaberrima*) African rice, Ta et al (2017) found in *O. Glaberrimaa*
689 wider rachis meristem associated with a more branched panicle. In fact, correlations between
690 apical meristem and leaf size were widely reported: according to Itoh et al. (2005), the apical
691 meristem size increased with leaf rank, generating longer and wider leaves; Fiorani et al.
692 (2000) observed leaf dimensions and elongation rate increased with meristem size; and
693 Lacube et al. (2017) found in maize that meristem size drove leaf width. These results
694 suggested that meristem size and activity conditioned morphogenesis (leaf dimension) and
695 organogenesis (branch and spikelet number) of initiated organs. The maintenance of flag
696 leaf width under water deficit is then an indicator of the apical meristem size maintenance
697 at early stage of the reproductive phase and thus of the ability of the plant to maintain high
698 branch and spikelet number on the panicle.

699 A comprehensive representation of tolerance to water deficit relies then on the
700 relationship between variations in flag leaf width and in branch number, as shown in Figure
701 7. Here, the overall tolerance of Cirad 409 and the overall sensitivity of IAC 25 were
702 reported. More, stem elongation rate was maintained with Cirad 409 under both climate
703 conditions, supporting the sink theory, as expressed by (Tardieu et al. 2018). In addition, the
704 response to water deficit of these two genotypes, as well as of HD 1-4 and Guarani, was
705 consistent across trials, through contrasted conditions of incoming radiation and
706 temperature, which highlighted the validity of this response over a large range of conditions.
707 Oppositely, the response of the other two genotypes, EM IAC 165 and Cirad 392, was not
708 consistent over the present climate conditions: these genotypes may be poorly adapted to
709 low radiation, or to high temperatures and VPD.

710 **Figure 7 -Relative variation of branch number and flag leaf width under water deficit.**

711 **Conclusions**

712 The plants grown in the two contrasted environments under study displayed
713 phenological and morphological differences at the time when water deficit was applied.
714 Considering these distinct initial status, plants reacted to water deficit with the same
715 processes, but with different intensity according to radiation and VPD conditions: the
716 increase in phyllochron, and in soluble sugar content in source and sink organs, were
717 associated with the decrease in stem elongation rate. Across both environments, this unique
718 response did not have the same impact on the performance of yield components (branch and
719 spikelet number on the panicle) with respect to genotypes, and could explain why a genotype
720 is adapted or not. This highlights the necessity to restrict the validity of results and
721 interpretation to the conditions explored by the study.

722 One key methodological practice here during the reproductive phase was to
723 identify elementary traits that are actually affected by the transient water deficit and to
724 conduct phenotyping only on these traits. Considering the diversity within phenological age
725 across genotypes during the reproductive phase, it was necessary for this purpose to map
726 panicle initiation respective of each genotype. Also in this study, it was a central hypothesis
727 to consider that the most tolerant genotypes were those minimizing the effect of the transient
728 water deficit on growth processes.

729 The flag leaf width finally appeared as a key trait to detect tolerance in case of a
730 water deficit occurring at the early and medium stages of the reproductive phase. In both
731 environments tested in this study, flag leaf width reduction in response to water deficit was
732 highly correlated with the reduction in branch and spikelet number in the panicle of the same
733 tiller. The reduction in flag leaf width was already reported in the literature as being a solid
734 indicator of the decrease in size and activity of the apical meristem. We emphasize here that
735 other traits could also be used for screening for drought tolerance according to the exact
736 timing of water stress, like the variation in the diameter of an internode or the peduncle. The

737 advantage to target such “secondary traits” resides in their capacity (i) to quantify precisely
738 the genotype-based effect of water deficit and so to distinguish the stress response across
739 genotypes and (ii) to detect relevant QTLs, closer from the basic metabolic processes that
740 drive rice plant response to water deficit.

741 **List of abbreviations**

742 TPE: Target populations of environment

743 HFE: Highly favorable environment

744 FE: Favorable environment

745 LFE: Least favorable environment

746 QTL: Quantitative trait loci

747 PI: Panicle initiation

748 SITIS: Integrated System for Induced Treatment of Drought

749 PVC: Polyvinyl chloride

750 IRR: Fully irrigated

751 STR: Water stress

752 NPK: Nitrogen phosphorus ans potassium

753 FC: Field capacity

754 WP: Wilting point

755 FTSW: Fraction of transpirable soil water

756 T min: Minimal temperature

757 T max: Maximal temperature

758 Rad: Daily cumulated incident radiation

759 VPD: air vapor pressure deficit

760 Tb: Base temperatura

761 Topt1: Temperature optimum 1

762 Topt2: Temperature optimum 2

763 HI: Haun Index

764 DAP: Days after planting

765 FLNb: Flag leaf number

766 RGR: Relative growth rate

767 GR: Growth rate

768 Exp.: Experiment

769 Trmnt: Treatment

770 IN: Internode

771 Max: Maximum

772 vs: versus

773 PCA: Principal component analysis

774 ΔGrowthRate: Relative variation of growth rate between IRR and ST treatments

775 ΔElongRate: Relative variation of total elongation rate between IRR and ST treatments

776 ΔTillNb: Relative variation of total tiller number between IRR and ST treatments

777 ΔFLLength: Relative variation of total flag leaf length between IRR and ST treatments

778 ΔFLWidth: Relative variation of total flag leaf width between IRR and ST treatments

779 ΔIN2Length: Relative variation of total internode 2 length between IRR and ST treatments

780 ΔIN2Width: Relative variation of total2 width between IRR and ST treatments

781 ΔLeafTransp: Relative variation of total leaf transpiration between IRR and ST treatments

782 ΔFLSolSug: Relative variation of total flag leaf soluble sugars between IRR and ST

783 treatments

784 ΔFLStarch: Relative variation of total flag leaf starch between IRR and ST treatments

785 ΔIN2SolSug: Relative variation of total internode 2 soluble sugar between IRR and ST

786 treatments

ΔIN2Starch: Relative variation of total internode 2 starch between IRR and ST treatments

ΔPhyllochron: Relative variation of total phyllochron between IRR and ST treatments

ΔTotalBranchNb: Relative variation of total branch number between IRR and ST treatments

ΔSpikeletNb: Relative variation of total spikelet number between IRR and ST treatments

TillNb: Tiller number

FLLength: Flag leaf length

FLWidth: Flag leaf width

IN2Length: Internode 2 length

IN2Width: internode 2 width

TotalBranchNb: Total branch number

SpikeletNb: Spikelet number

ElongRate: Elongation rate

FLSolSug: Flag leaf soluble sugars content

FLStarch: Flag leaf starch content

IN2SolSug: Internode 2 soluble sugars content

IN2Starch: Internode 2 starch content

LeafTransp: Leaf transpiration rate

PQ: Photothermal quotient

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

812 All data generated or analysed during this study are included in this published article.

813 **Competing interests**

814 The authors declare that they have no competing interests

815 **Funding**

816 This research received funding 1) from Agropolis Foundation and Embrapa through the
817 DRYCE project for the experiments conducted with the japonica panel and 2) from Capes
818 for the scholarship of the first author.

819 **Authors' contributions**

820 APdC and TL coordinated the Dryce research project.IPdL, MdR, and APdC designed and
821 carried out the Sitis experiment in Goiania. IPdL, SR, MdR and TLdesigned and carried out
822 the growth chamber experiment in Montpellier. ACV and AS performed non-structural
823 carbohydrate measurements and their statistical analysis. IPdL and MdR, with help of FB
824 and TL, performed the data analysis and interpretation.MdR, IPdL and TL wrote the
825 paper,which was edited and approved by all co-authors.

826 **Acknowledgements**

827 The authors thank the Agropolis Foundation (France) and Embrapa (Brazil) for funding the
828 bilateral DRYCE project and Capes (Brazil) for supporting Isabela's PhD scholarship. The
829 authors would like to thank all the Embrapa staff who contributed to the experiment, with
830 particular thanks to Cleicomar Gonçalves de Almeida for help in conducting the experiment
831 in the Sitis platform. Finally, we would like thank to Lauriane Rouan and Denis Cornet
832 (Cirad) for help in the statistical analyses.

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Table 6 – Impact of water deficit on reproductive phase processes in Sitis.

% of time process affected by water deficit										
SITIS										
Genotypes	**Panicle primordium formation**	**Flag leaf formation**	**Lower Internode elongation**	**Branching I formation**	**Branching II formation**	**Internode 2 elongation**	**Spikelet formation**	**Internode 1 elongation**	**Pollen viability**	**Peduncle elongation**
CIRAD 409	0	0	0	0	0	0	73	89	100	100
HD 1-4	0	0	0	23	33	33	73	100	100	100
CIRAD 392	0	0	0	30	38	38	94	100	100	100
IAC 25	0	0	0	30	38	38	94	100	100	100
EM IAC 165	0	0	0	36	44	44	94	100	100	100
GUARANI	0	0	0	45	63	63	100	100	100	100

Table 7 – Impact of water deficit on reproductive phase processes in Growth Chamber.

% of time process affected by water deficit										
Growth chamber										
Genotypes	**Panicle primordium formation**	**Flag leaf formation**	**Lower internode elongation**	**Branching I formation**	**Branching II formation**	**Internode 2 elongation**	**Spikelet formation**	**Internode 1 elongation**	**Pollen viability**	**Peduncle elongation**
IAC 25	0	38	38	100	100	100	100	100	100	100
EM IAC 165	10	50	50	100	100	100	100	100	100	100
CIRAD 392	23	62	62	100	100	100	100	100	100	100
HD 1-4	44	69	69	100	100	100	100	100	100	100
GUARANI	57	79	79	100	100	100	100	100	100	100
CIRAD 409	64	84	84	100	100	100	100	100	100	100

Table 11 – Correlations matrix of the studied variables.

Variables	ΔGrowth rate	ΔElong rate	ΔTiller Nb	ΔFL length	ΔFL width	ΔIN2 length	ΔIN2 width	ΔLeafTransp	ΔFlagLeaf SolSug	ΔFlag LeafStarch	ΔIN2 SolSug	ΔIN2 Starch	ΔPhyllochron	ΔTotal BranchNb
ΔGrowthRate	**1**													
ΔElongRate	0.218	**1**												
ΔTillNb	0.455°	-0.049	**1**											
ΔFLLength	-0.209	-0.204	-0.369	**1**										
ΔFLWidth	-0.196	-0.054	-0.521	**0.580***	**1**									
ΔIN2Length	0.412	**0.869*****	-0.129	-0.301	-0.050	**1**								
ΔIN2Width	0.149	-0.001	-0.498	0.435	**0.712****	0.266	**1**							
ΔLeafTransp	-0.188	0.291	-0.419	0.131	0.044	0.438	0.396	**1**						
ΔFLSolSug	-0.188	**-0.659***	-0.329	0.447	0.220	**-0.594***	0.239	-0.194	**1**					
ΔFLStarch	0.222	**0.686***	-0.425	-0.141	0.329	**0.769****	0.482	0.402	-0.195	**1**				
ΔIN2SolSug	0.287	-0.518°	-0.009	0.151	-0.052	-0.209	0.458	0.097	**0.593***	-0.055	**1**			
ΔIN2Starch	0.203	0.453	0.218	-0.254	-0.322	0.434	0.063	0.217	-0.221	0.370	0.305	**1**		
ΔPhyllochron	0.045	**-0.759****	-0.073	-0.117	-0.006	-0.401	0.193	-0.124	0.376	-0.318	**0.644***	-0.220	**1**	
ΔTotal BranchNb	-0.178	0.047	-0.511°	**0.730****	**0.736****	0.032	**0.728****	0.526°	0.207	0.375	0.193	0.025	-0.136	**1**
ΔSpikeletNb	-0.160	0.209	-0.470	**0.712****	**0.703***	0.155	**0.619***	**0.578***	0.071	0.413	-0.036	-0.064	-0.331	**0.945*****

°:Significant at P< 0.1; *: significant at P < 0.05; **: significant at P < 0.01; and ***: significant at P < 0.001.

Table 12 – Response index in Sitis and in Growth Chamber experiments for all studied traits and genotypes.

	CIRAD 392		CIRAD 409		EM IAC165		GUARANI		HD1-4		IAC25	
Traits	**SITIS**	**Growth Chamber**	**SITIS**	**Growth Chamber**	**SITIS**	**Growth Chamber**	**SITIS**	**Growth Chamber**	**SITIS**	**Growth Chamber**	**SITIS**	**Growth Chamber**
ΔTillNb	-0.12^{ns}	0.13^{ns}	0.04^{ns}	-0.26^{ns}	-0.10^{ns}	0.26^{ns}	-0.13^{ns}	0.06^{ns}	-0.07^{ns}	-0.11^{ns}	-0.27*	-0.06^{ns}
ΔPlant Height (mm)	-0.26**	-0.23**	-0.03^{ns}	0.02^{ns}	-0.28**	-0.18**	-0.21**	-0.12*	-0.15*	-0.19**	-0.20**	-0.19**
ΔPlant Biomass (g)	-0.14^{ns}	-0.40**	-0.01^{ns}	0.04^{ns}	-0.16^{ns}	0.31*	-0.19^{ns}	-0.15^{ns}	-0.21*	-0.36*	-0.38**	-0.21^{ns}
ΔGrowth Rate (g.day^{-1})	-0.15	-0.39**	-0.01^{ns}	0.03^{ns}	-0.16^{ns}	0.41*	-0.19^{ns}	-0.17^{ns}	-0.21*	-0.38*	-0.38**	-0.21^{ns}
ΔFLLength (mm)	0.16^{ns}	-0.17^{ns}	0.03^{ns}	-0.15^{ns}	-0.16^{ns}	-0.25^{ns}	0.00	-0.18^{ns}	-0.02^{ns}	-0.13^{ns}	-0.09^{ns}	-0.16^{ns}
ΔFLWidth (mm)	0.08^{ns}	0.00	0.04^{ns}	0.07^{ns}	0.03^{ns}	-0.17**	-0.02^{ns}	-0.12^{ns}	-0.07^{ns}	-0.11^{ns}	-0.03^{ns}	-0.05^{ns}
ΔIN2Length (mm)	-0.39*	-0.34*	-0.04^{ns}	0.21^{ns}	-0.32*	-0.12^{ns}	-0.26*	-0.06^{ns}	-0.27*	-0.13^{ns}	-0.22*	-0.28**
ΔIN2Width (mm)	-0.03^{ns}	-0.18**	0.00	0.01^{ns}	0.03^{ns}	-0.17**	-0.03^{ns}	-0.09^{ns}	-0.14*	-0.16* ^{n}	-0.09^{ns}	0.16**
ΔTotalBranchNb	0.09^{ns}	-0.31*	-0.07^{ns}	-0.08^{ns}	-0.12^{ns}	-0.46**	-0.13^{ns}	-0.19^{ns}	-0.23*	-0.17^{ns}	-0.31**	-0.25^{ns}
ΔSpikeletNb	0.06^{ns}	-0.28*	-0.01^{ns}	-0.03^{ns}	-0.17*	-045**	-0.19*	-0.24^{ns}	-0.17*	-0.10^{ns}	-0.33**	-0.26*
ΔElongRate (mm.day^{-1})	-0.44**	-0.34**	-0.20^{ns}	-0.01^{ns}	-0.55**	-0.33**	-0.38**	-0.17*	-0.31*	-0.26**	-0.41**	-0.28**
ΔFLSolSug (mg.g^{-1})	0.90**	0.26^{ns}	-0.02^{ns}	0.18^{ns}	0.82**	0.27*	0.66**	0.29*	0.97**	0.06^{ns}	0.68**	0.15^{ns}
ΔFLStarch (mg.g^{-1})	-0.18**	-0.43^{ns}	-0.28**	1.16^{ns}	-0.13^{ns}	-0.38^{ns}	-0.34**	0.14^{ns}	-0.27**	-0.18^{ns}	-0.39**	-0.37^{ns}
ΔIN2SolSug (mg.g^{-1})	1.48**	-0.62*	0.08^{ns}	-0.02^{ns}	1.91**	1.21*	1.10**	1.40*	0.38^{ns}	-0.04^{ns}	0.85**	-0.35^{ns}
ΔIN2Starch (mg.g-1)	-0.06^{ns}	-0.34^{ns}	-0.01^{ns}	0.17^{ns}	-0.17^{ns}	0.09^{ns}	-0.26**	1.47^{ns}	-0.17^{ns}	-0.19^{ns}	-0.20*	-0.06^{ns}
ΔLeafTransp (mmol.m^{-2}.s^{-1})	-0.17^{ns}	-0.36**	-0.05^{ns}	-0.05^{ns}	0.06^{ns}	-0.31*	-0.15^{ns}	-0.05^{ns}	-0.17*	0.15^{ns}	-0.20*	-0.25*

Response index represents the relative variation of a trait between stressed and control treatments (see Mat 1 Meth); negative value means a reduction in stressed plants in relation with control ones. **: significant by F test at 1% probability; *: significant by F test at 5%; ^{ns}: not significant.

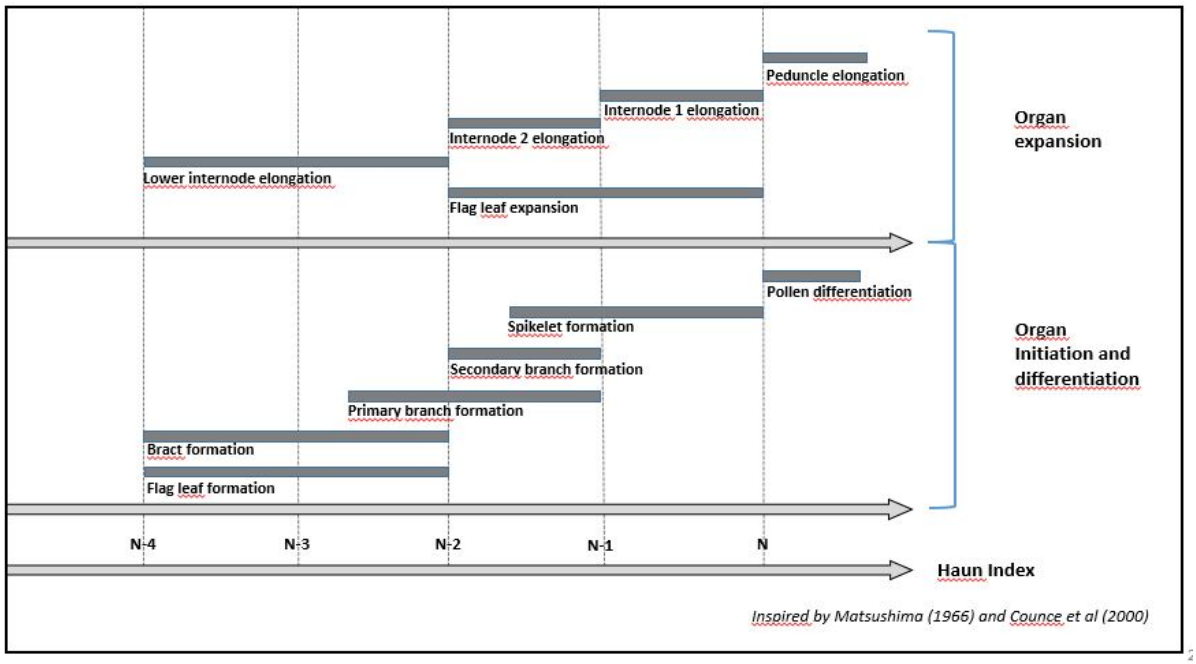


Figure 1- Timing of developmental processes during the reproductive phase in rice

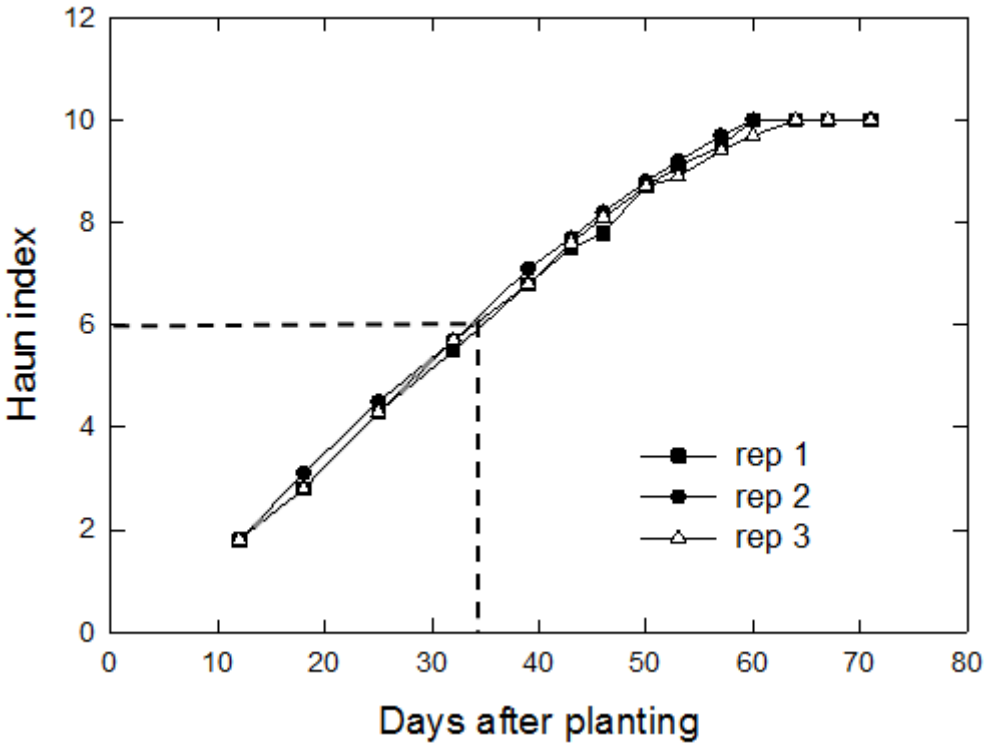


Figure 2 - Determination of the panicle initiation date on irrigated Cirad 409 in the growth chamber experiment

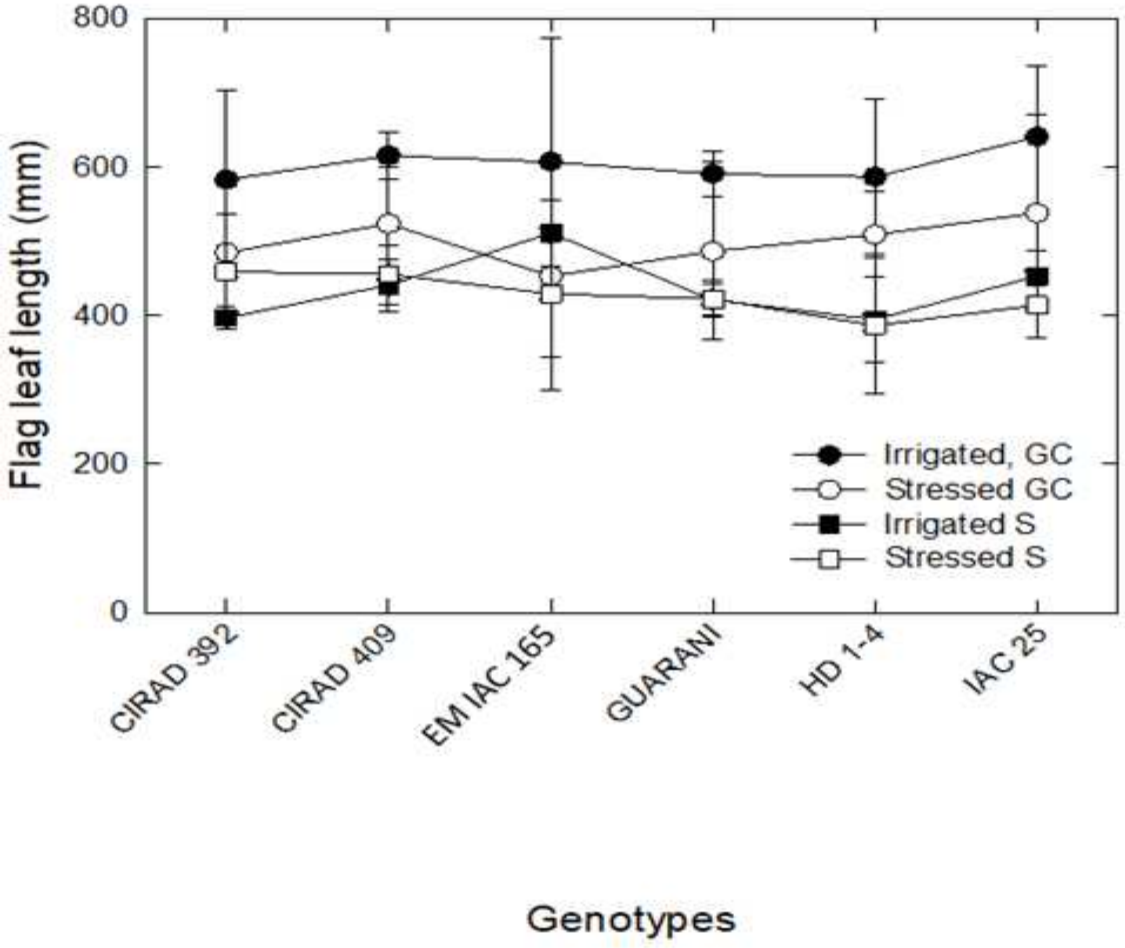


Figure 3 - Flag leaf length in relation with the experiments and water treatments.

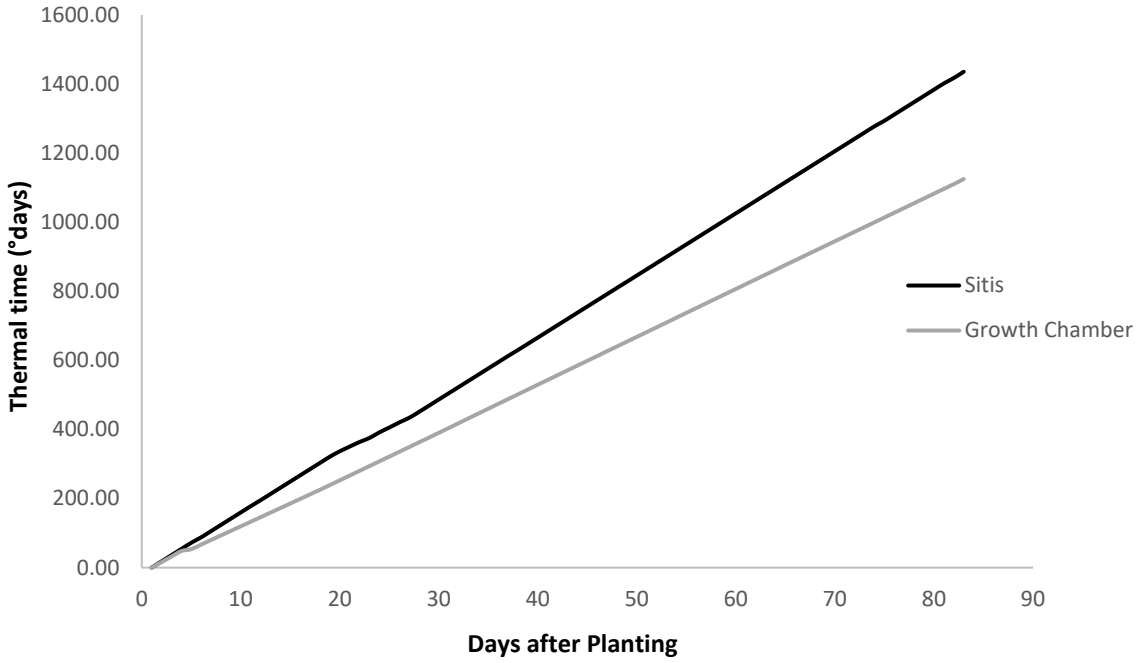


Figure 4 - Cumulated thermal time in Sitis and growth chamber experiments in function of calendar time.

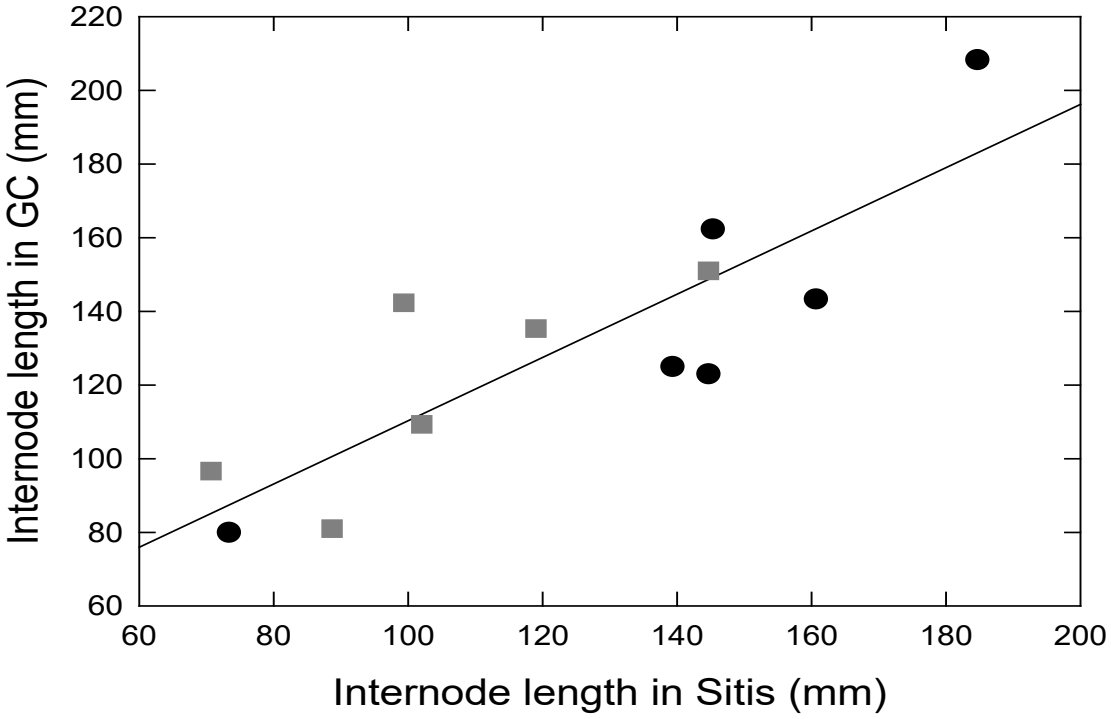


Figure 5 - Relationship between internode 2 length in Growth Chamber and Sitis experiments

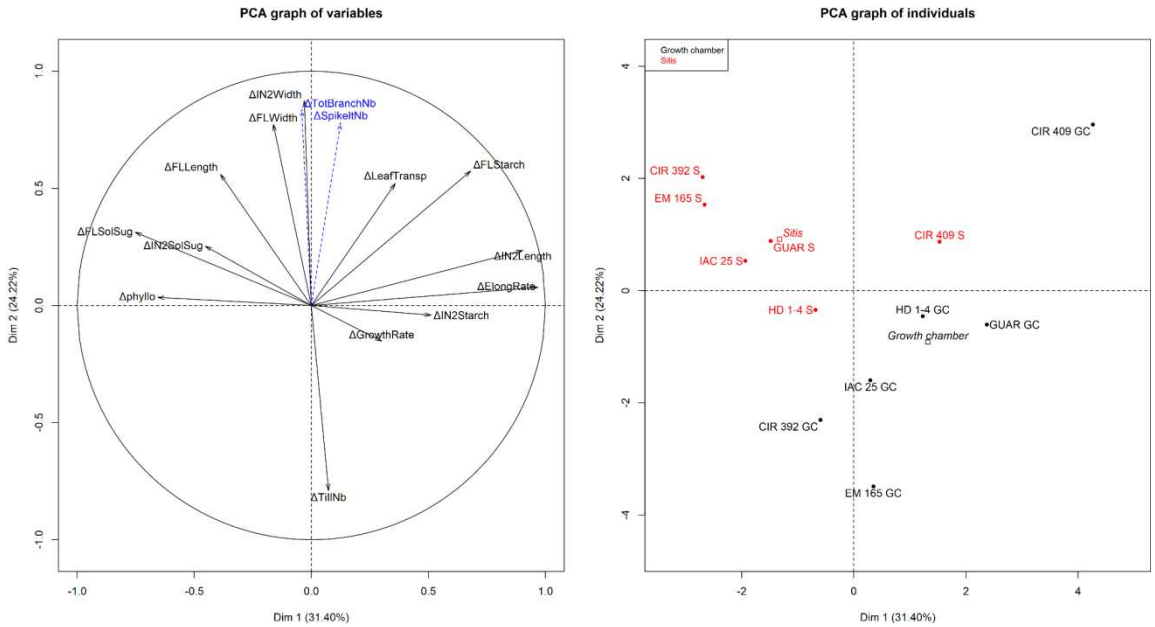


Figure 6 - Principal Component Analysis representation on the two first components of the response index.

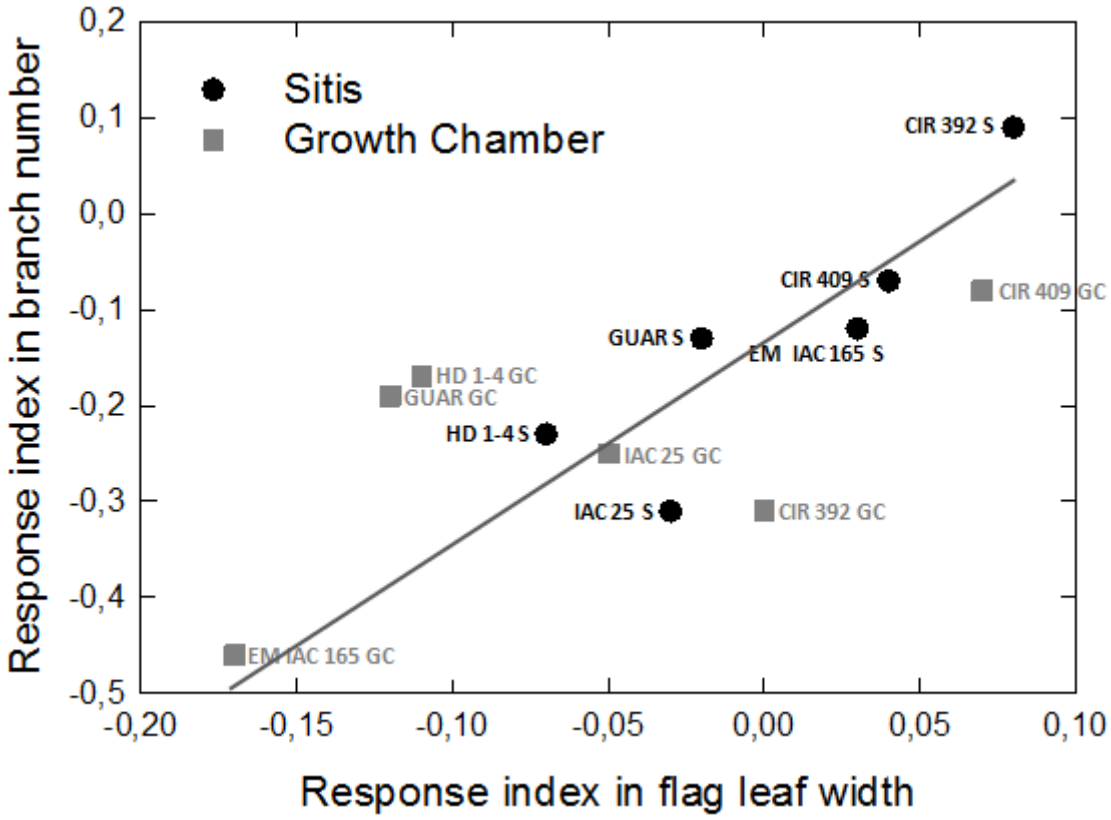


Figure 7 - Relative variation of branch number and flag leaf width under water deficit.

Figures

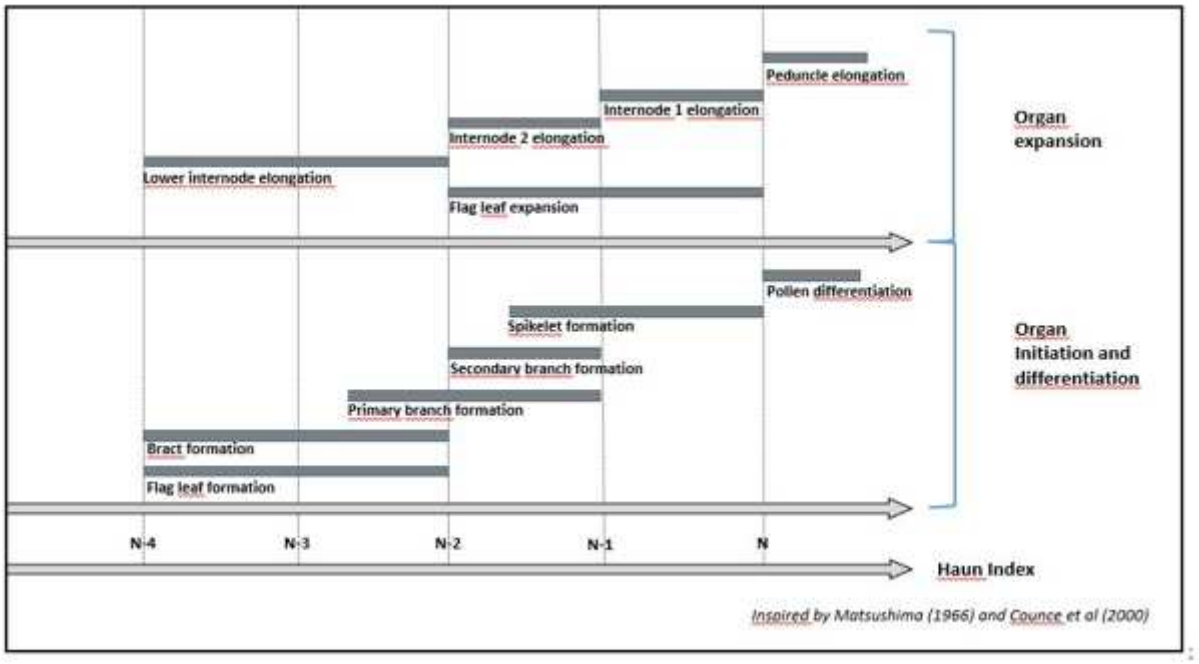


Figure 1

Timing of developmental processes during the reproductive phase in rice

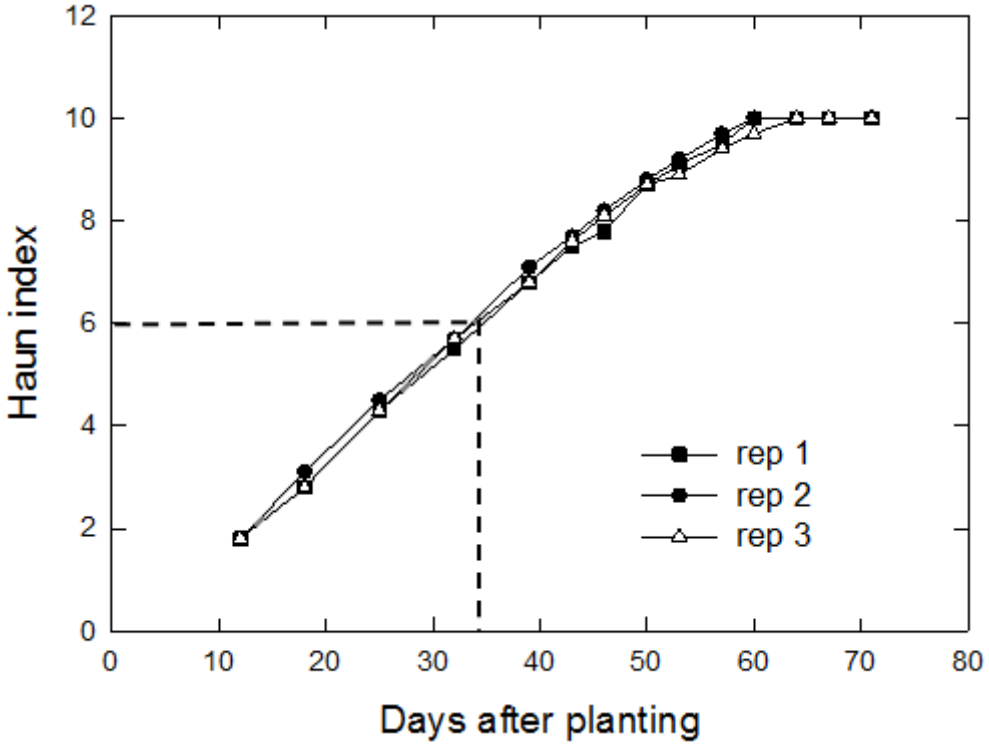
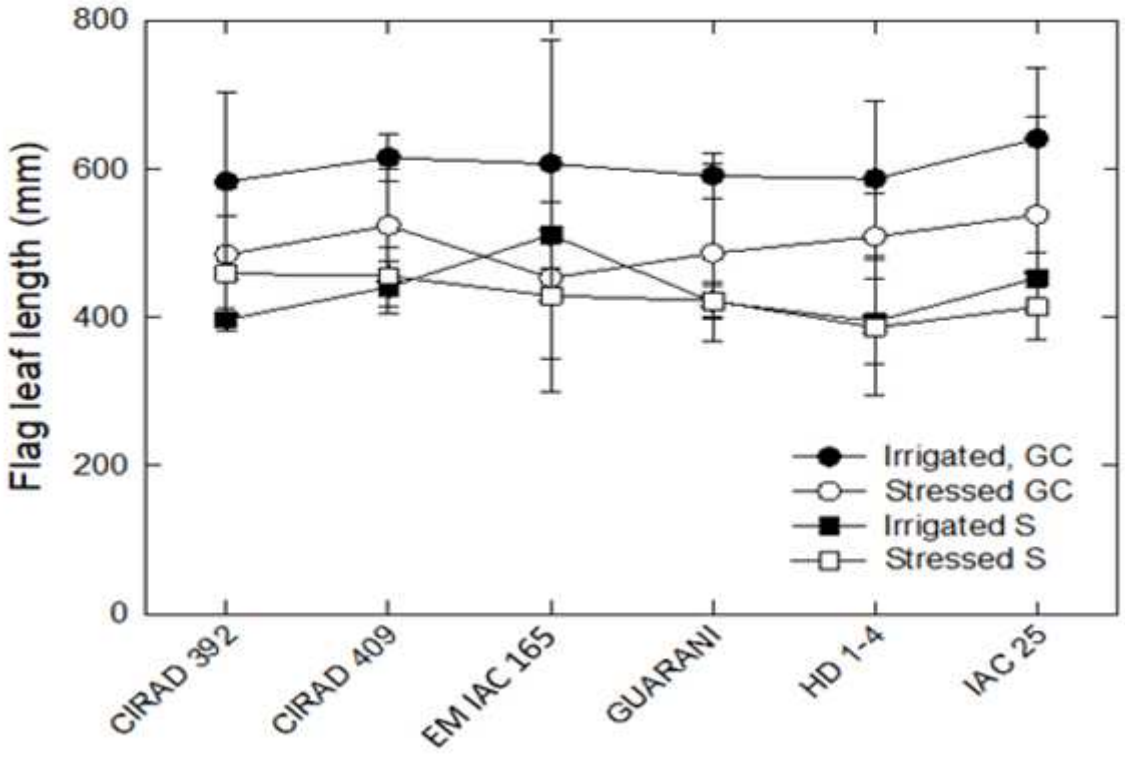


Figure 2

Determination of the panicle initiation date on irrigated Cirad 409 in the growth chamber experiment



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Flag leaf length in relation with the experiments and water treatments.

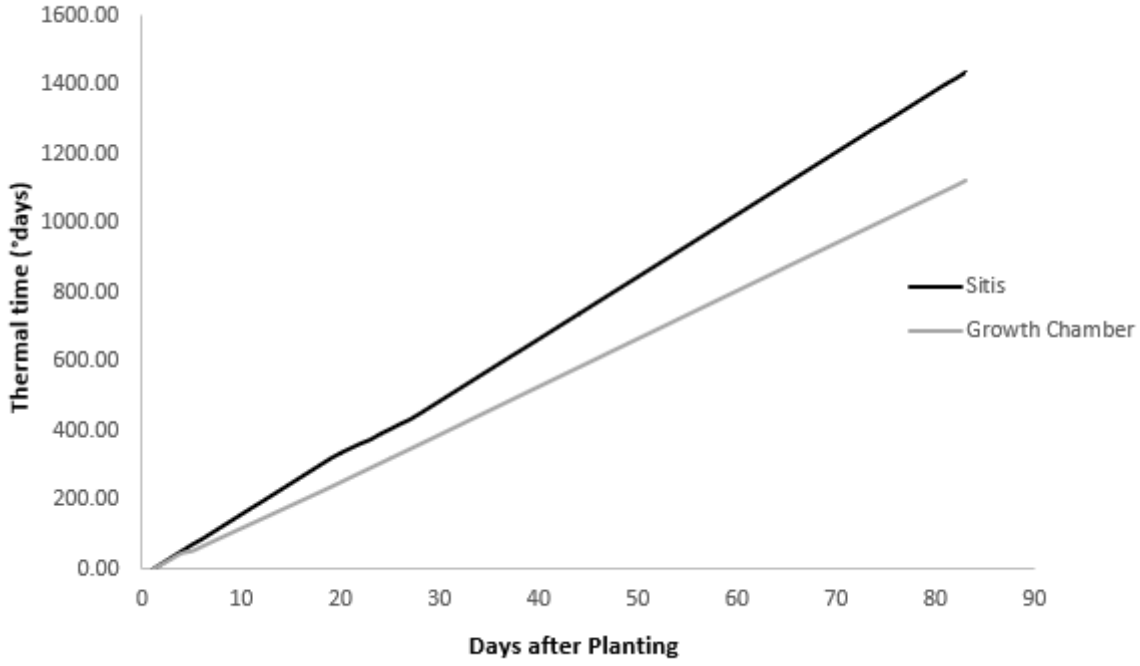

Figure 4

Cumulated thermal time in Sitis and growth chamber experiments in function of calendar time.

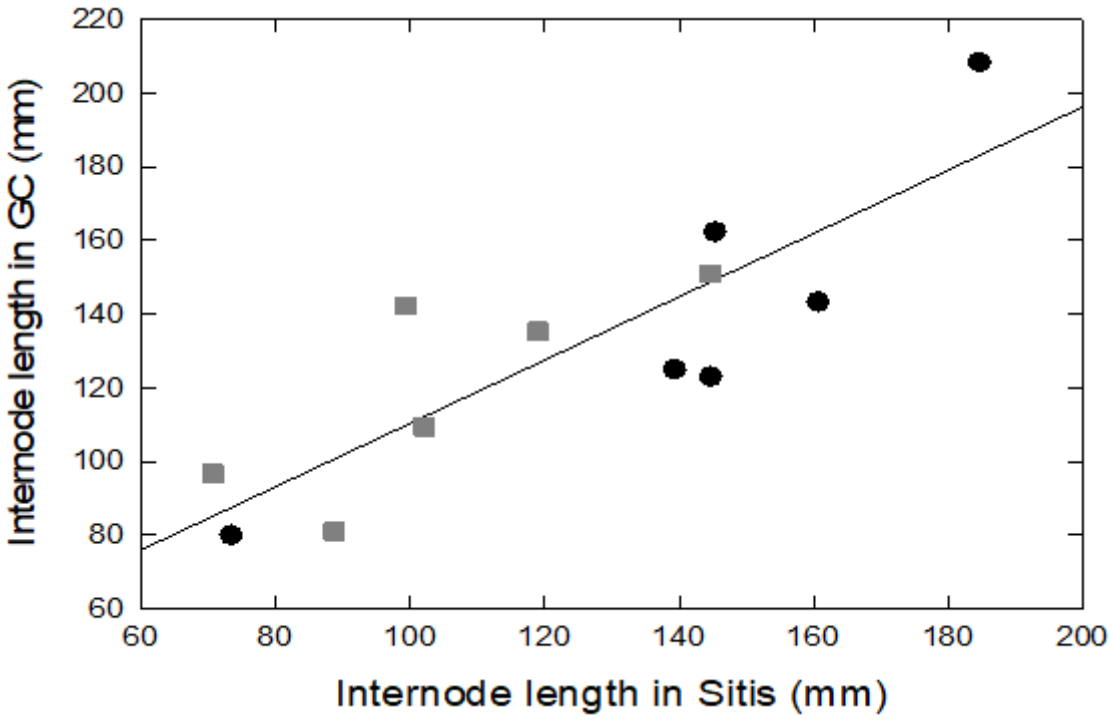

Figure 5

Relationship between internode 2 length in Growth Chamber and Sitis experiments

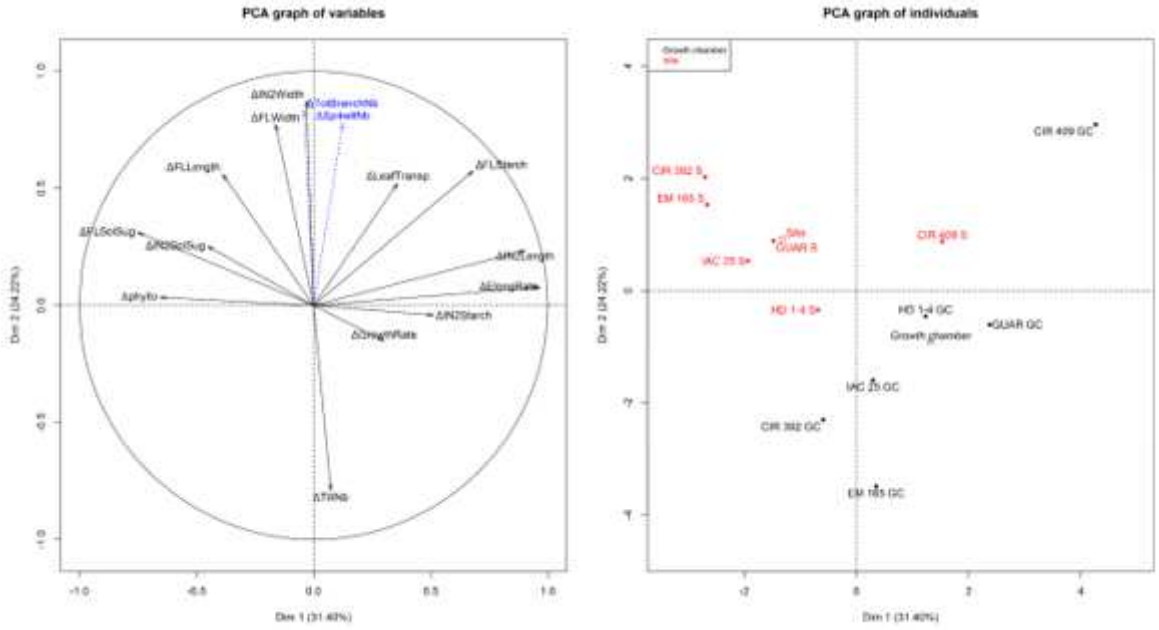

Figure 6

Principal Component Analysis representation on the two first components of the response index.

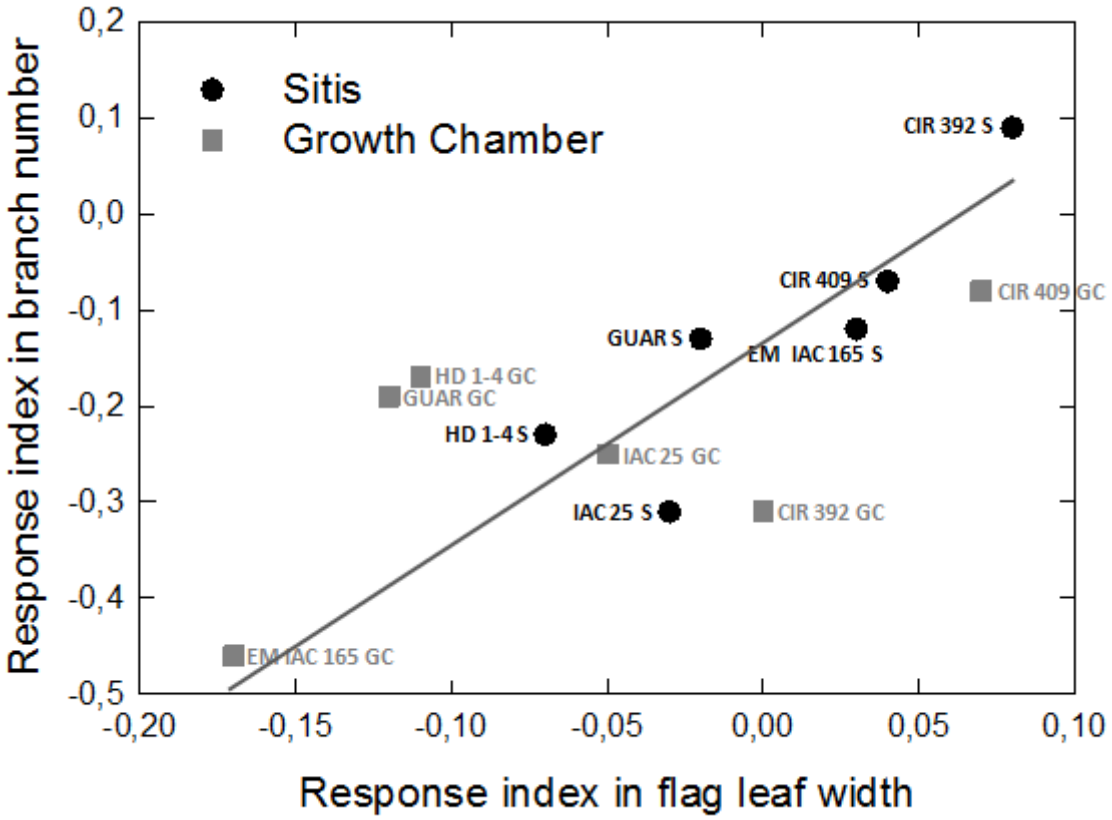

Figure 7

Relative variation of branch number and flag leaf width under water deficit.