

Rice growth under water stress levels imposed at distinct developmental stages

Crescimento do arroz sob níveis de estresse hídrico impostos em diferentes estádios de desenvolvimento

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S U M M A R Y

We aimed to assess rice growth as function of water stress at distinct crop stages, under greenhouse, using randomized block design and factorial scheme $3 \times 4 + 1$, with four replications. Factor "A" was the growth stage when water stress was imposed, being (1) vegetative, (2) reproductive 1, and (3) reproductive 2; factor "B" was four levels of water stress (0 -200 kPa). The additional treatment consisted of a flooded check. Water was replenished to saturation every time the threshold stress was reached. Rice height is reduced when stress is applied either at vegetative or reproductive stages; tillering was affected only by water stress at the vegetative stage, and plants did not recover after the stress was withdrawn; surplus in leaf area is likely to help recovering plant performance from stress applied at the vegetative stage; water content was not affected by water stress, but shoot dry mass of culms was reduced with stress between tillering start and anthesis; root volume did not increase as function of water stress at the vegetative stage. Rice growth is little affected by the absence of waterlogging, but stress levels should be below 30 kPa throughout cropping cycle.

Keywords: *Oryza sativa*, phenology, water demand.

R E S U M O

Avaliou-se o crescimento do arroz em função do estresse hídrico em diferentes fases fenológicas, em casadevegetação, em delineamento de blocos casualizados, esquema fatorial $3 \times 4 + 1$, com quatro repetições. O fator "A" representou a fase fenológica da planta com imposição de estresse, sendo (1) vegetativo, (2) reprodutivo 1, (3) reprodutivo 2; o fator "B" representou os níveis de estresse (0 -200 kPa); o tratamento adicional foi composto por uma testemunha inundada. A água foi repostada ao alcançar o nível de estresse determinado para o tratamento. As plantas cresceram menos com estresse tanto nas fases vegetativa como reprodutiva; o perfilhamento foi afetado pelo estresse na fase vegetativa; a expansão de área foliar auxilia na posterior recuperação da planta quando o estresse foi imposto na fase vegetativa; o conteúdo de água não foi afetado pelo estresse, mas a massa seca de colmos foi reduzida quando o estresse ocorreu entre início do perfilhamento e anese; por fim, o volume de raízes não aumentou com imposição de estresse na fase vegetativa. O crescimento do arroz é pouco afetado pela falta de inundação, mas o estresse hídrico deve permanecer abaixo de 30 kPa durante todo o ciclo da cultura.

Palavras-chave: *Oryza sativa* L., fenologia, demanda hídrica.

INTRODUCTION

Rice is a staple food for nearly half the world's population, being cultivated in 112 countries, with 90% of the world's production concentrated in Asia. In Brazil, about 3 million hectares are cultivated every year and rice is traditionally present in Brazilian meals, regardless of social class. The southern region of the country supplies approximately 65% of Brazilian rice (Gomes and Magalhães Jr., 2004). The demand for water in flooded rice cultivation is considerably higher than the water requirement of crops traditionally sprinkler irrigated, such as soybeans and corn. This raises a series of questions, ranging from water use efficiency to environmental issues when growing rice. Physiologically, this species is a sub-aquatic plant adapted to a flooded environment (Correll and Correll, 1975), being thus possible to be grown in absence of waterlogging.

Sprinkler irrigation by pivot and linear system has been, and continues to be, tested for rice cultivation, and there are claims for 50% water savings when rice grown under pivot irrigation is compared to continuous flooding (Parfitt *et al.*, 2011). This seems to be the case mainly when the system is installed in uneven areas or in fields with significant slope, as well as, where water is scarce. Supposing this economy is confirmed, farmers who grow rice under pivots would have a surplus of water that could be used either to increase rice acreage or to irrigate crops on additional fields.

In order to support rice-growing systems which demand less water and help environmental conservation, there is a need to understand how rice plants are affected by, and respond to, distinct levels of water stress imposed at different crop phenological stages. This knowledge is the base, which paves the way for the planning of proper, low-cost and efficient rice production systems while reducing the environmental impact in its production.

Water content in plants is important for keeping hydration, maintaining cell turgidity for structure and growth; it is also greatly responsible for the transport of nutrients and organic compounds into the plant, and also serves as raw material for chemical processes and buffers the plant against

temperature oscillations (Gurevitch *et al.*, 2009). As rice is usually grown under waterlogged soils, there was little need in the past to understand the effects of drought in rice development. When rice passed to be grown under sprinkler irrigation in highland soils, water stress started to be significant.

Besides water content, rice plant height, tillering vigor and leaf area are major factors responsible for its competitiveness against important weed species (Aldrich, 1984). For a crop to become successful, it should germinate and emerge fast after planting, and occupy the area as rapidly as possible to avoid the establishment of other competitor plants (Gurevitch *et al.*, 2009).

This study aimed to assess rice growth parameters as function of water stress levels imposed at distinct stages of crop development.

MATERIALS AND METHODS

The experiment was installed in a greenhouse at Embrapa Clima Temperado, Pelotas-RS, Brazil, during the traditional rice-growing season. A randomized complete-block design with plots arranged in a factorial scheme, $3 \times 4 + 1$, with four replications was used. The rice variety was BRSQuerencia, with early cycle duration (Embrapa, 2005). Factor "A" comprised the growth stage when water stress was imposed on the treatments, being (1) vegetative (from the beginning of tillering to panicle differentiation), (2) reproductive 1 (from panicle differentiation to anthesis), and (3) reproductive 2 (from anthesis to the beginning of ripening). Factor "B" comprised the four levels of water stress imposed to the plants. The additional treatment consisted of a constantly flooded treatment (Table 1).

Although the reproductive stage in rice starts at panicle initiation (SOSBAI, 2016), this stage is very difficult to identify; as a result, farmers generally use panicle differentiation as the start of the reproductive stage for nutrient management. As panicle initiation (PI) and panicle differentiation (PD) are spaced only in about four days (Carli *et al.*, 2016), panicle differentiation was used in the present study.

From emergence to the beginning of tillering, all plots were maintained with soil water tension less than 10 kPa – including plots which would be flooded from tillering onward. Every time the treatment reached the threshold level of water deficit, it was irrigated back to saturation. Treatments that were not at the developmental stage when the stress was previewed to be applied were maintained under 10 kPa. Treatments are listed in Table 1.

Experimental units consisted of black plastic pots with a capacity of 12 L, filled with 10 kg of previously corrected and fertilized soil. The soil used at the experiment was collected in agriculture-free natural areas near rice fields at Terras Baixas Experimental Station, Capão do Leão, RS, Brazil. Soil was fertilized with NPK and corrected for pH 6.0 with ground limestone. In rice fields, pH is usually not corrected because the water layer is enough to correct the pH after flooding is established, but as most plots of the trial were not going to be submitted to flooding, a correction of soil pH was done in order to guarantee equal soil pH conditions for all plots. Seven rice seeds were sown into

each experimental unit, and after emergence the five most homogeneous plants were maintained.

Water stress was monitored by using sets of Watermark (model 200SS) electro-tensiometers (Irrometer Inc., Riverside, CA), with a single sensor installed in each experimental unit, at depth of 10 cm (from soil surface to the center of the sensor), at the radial center of the pot. All sensors were connected by wire to a nearby Watermark data logger, which was programmed to record water tension in kPa at one-hour intervals. The data logger automatically corrected sensor readings as a function of the mean temperature registered inside the plots, and for that, two Watermark temperature sensors were installed in each block of the experiment. Temperature data from these sensors were used by the data logger to correct soil water tension readings of the corresponding plots. Temperature sensors were also installed at a depth of 10 cm.

Soil water tension for all plots was read and recorded manually, twice a day (09:00 am and 04:00 pm), seven days a week. When it reached the threshold level, the water needed to adjust soil

Table 1 - Treatments studied at the greenhouse trial

Treatment	Description / Details
Flooded	Flooded with 7 cm of water at the beginning of tillering and kept flooded until ripening start. At ripening start, water was not removed, and remained for some days before plots were dry.
	"V" – under treatment between the beginning of tillering and panicle differentiation
10 kPa	Irrigated back to the saturation every time water tension reached 10 kPa .
30 kPa	Irrigated back to the saturation every time water tension reached 30 kPa .
100 kPa	Irrigated back to the saturation every time water tension reached 100 kPa .
200 kPa	Irrigated back to the saturation every time water tension reached 200 kPa .
	"R1" – under treatment between panicle differentiation and anthesis
10 kPa	Irrigated back to the saturation every time water tension reached 10 kPa .
30 kPa	Irrigated back to the saturation every time water tension reached 30 kPa .
60 kPa	Irrigated back to the saturation every time water tension reached 60 kPa .
130 kPa	Irrigated back to the saturation every time water tension reached 130 kPa .
	"R2" – under treatment between anthesis and ripening start
10 kPa	Irrigated back to the saturation every time water tension reached 10 kPa .
30 kPa	Irrigated back to the saturation every time water tension reached 30 kPa .
60 kPa	Irrigated back to the saturation every time water tension reached 60 kPa .
130 kPa	Irrigated back to the saturation every time water tension reached 130 kPa .

NOTE: In fact, treatments submitted to 10 kPa (V, R1 or R2) were under this water tension during all the cycle, because all treatments were kept between saturation and 10 kPa, when out of the developmental stage they were supposed to be under treatment.

water tension back to saturation was added after reading. The amount of water to be added to each plot was determined by using a soil moisture retention curve, which relates water tension (kPa) with water content (%). The water tension curve was determined especially for the experiment (Figure 1), after the soil was corrected and fertilized, so no error in the curve would be attributed to differential soil density or structure.

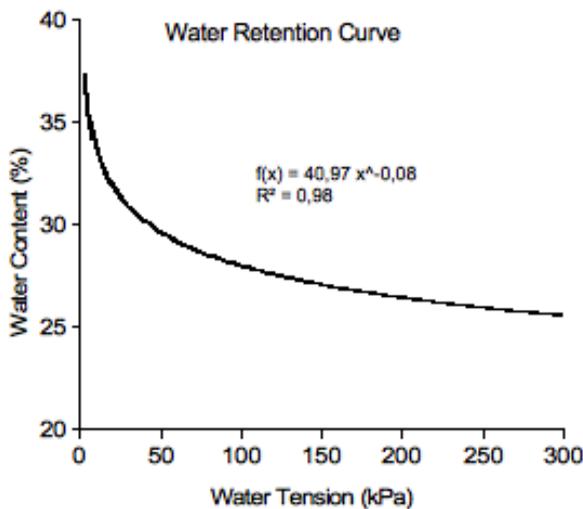


Figure 1 - Water retention curve for the soil used at the experiment under controlled environment (Embrapa Clima Temperado, Pelotas-RS, Brazil).

The daily maximum and minimum temperature and air humidity into the greenhouse during the application of the treatments are shown in Figures 2A and 2B, respectively. The evapotranspiration (ET_0) was calculated as an auxiliary method to the sensors supporting estimation of when plots would reach the threshold water stress level in order to avoid losing the timing for water application. ET_0 was calculated using the Hargreaves equation (Figure 2C), based on daily maximum and minimum temperatures (Figure 2A).

Weekly, starting from seven days after treatment establishment to the "V" group, the plant height, number of tillers per plant and leaf area were assessed. Plant height was evaluated by using a graduated ruler measuring the distance from the soil surface to the tip of the taller leaf; to access the number of tillers, tillers were counted in all plants, being presented as mean number of tillers per

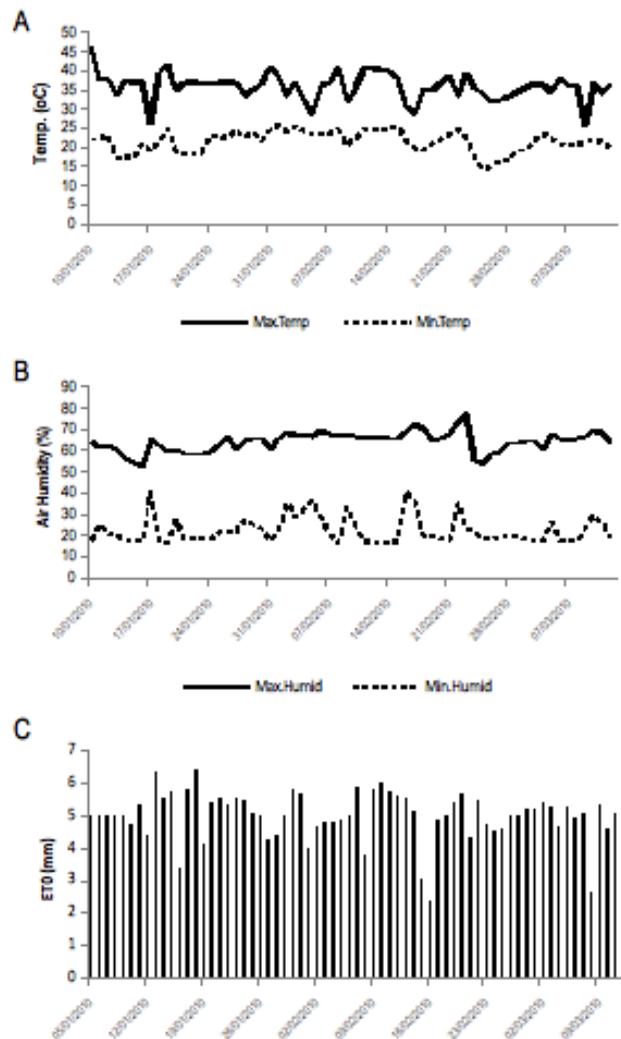


Figure 2 - (A) Daily maximum and minimum temperatures collected inside the greenhouse for ET_0 estimation; (B) maximum and minimum air humidity into the greenhouse throughout the experimental period; (C) ET_0 estimation by the Hargreaves formula based on maximum and minimum daily temperatures. Embrapa Clima Temperado, Pelotas-RS, Brazil, 2016.

plant; the leaf area was not directly measured but estimated, as this is a destructive parameter when directly read. For that, the length and width of all leaves per plant were measured, being the estimated leaf area obtained by the following formula (Carlesso *et al.*, 1998):

$$LA = \frac{\sum(l \times w) \times 0.75}{10,000}$$

where LA= (estimated) leaf area (m²); ll = leaf length (cm); lw = leaf width (cm).

At the end of the cycle, rice shoot water content (%), dry mass (g plant⁻¹) and root volume (cm³ plant⁻¹) were also assessed. Plants were cut at soil level, immediately weighed for fresh mass being later dried in air-circulated oven at 65 °C for four days, when the dry mass was evaluated. Shoot water content (%) was obtained by the following formula (Minshall, 1960):

$$WC = \frac{FW - DW}{FW} \times 100$$

where WC = water content (%); FW = fresh weight; DW = dry weight.

After collecting the plants, all the soil of each experimental plot was carefully washed under running water for obtaining the bare roots; the excess of water in roots was removed with absorptive paper towels by gently pressing and rubbing them against the roots until towels were almost dry after used. Roots were put into a graduated tube containing water, being root volume obtained by the difference between the water level before and after root immersion.

Data were analyzed into the “R” statistical environment (R Core Team, 2016). Before any analysis, data sets were verified for normality and variance homogeneity of residuals by the tests of ShapiroWilk and Bartlett, respectively, being transformed by when needed. Data were then submitted to analysis of variance by the Ftest at 5% probability. Data were explored according to significances of the interactions, being presented in graphical form. Data graphically presented are original; transformed data were used only for the parametric tests.

RESULTS

Plant height (Figure 3) was affected by water stress when it was imposed both at “V” and “R1” stages; as rice plants stop growing before “R2”,

differences due to stresses imposed at this stage were not expected. For the “V” stage, plants tended to be lower than the observed for the flooded check when water stress was beyond 100 kPa; 28 days after stress was imposed (after tillering start – DAT), plants at the two higher water tensions

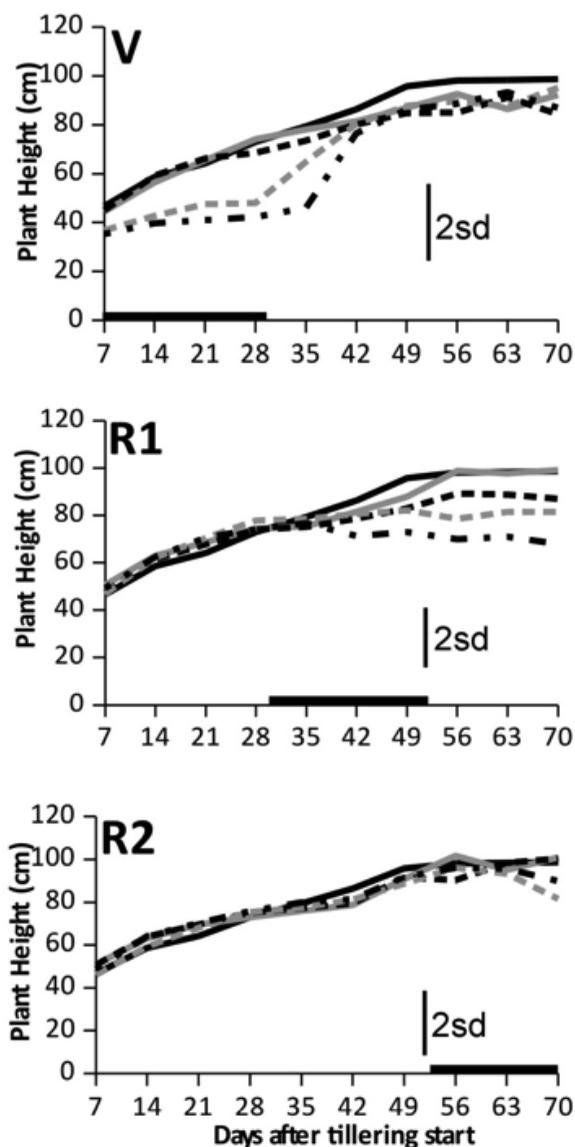


Figure 3 - Plant height (cm) as function of distinct water stress levels imposed to different developmental stages of rice plants cv. BRSQuerencia. V = vegetative stage (from tillering start to panicle differentiation); R1 = reproductive 1 (from panicle differentiation to anthesis); R2 = reproductive 2 (from anthesis to ripening start). (—) Flood / (---) 10kPa / (- - -) 30 kPa / (· · ·) 100V/60R kPa / (- · - ·) 200V/130R kPa.

measured about 40cm height, while those at the flooded check and also those whose water stress was up to 30 kPa, measured about 60 cm (Figure 3). The stress for the "V" stage was maintained for 30 days, and 42 DAT (12 days after stress was removed from "V" stage), plant height was recovered and no difference among treatments was later reported.

For "R1", only the stress level of 130 kPa caused reduction in plant height compared to the flooded check (Figure 3), and this was maintained to the end of the cycle; no growth recovery was observed when severe water stress was imposed between panicle differentiation and anthesis (R1). When stress was imposed at "R2", no damage to plant height was reported, since plants had already stopped growth at all.

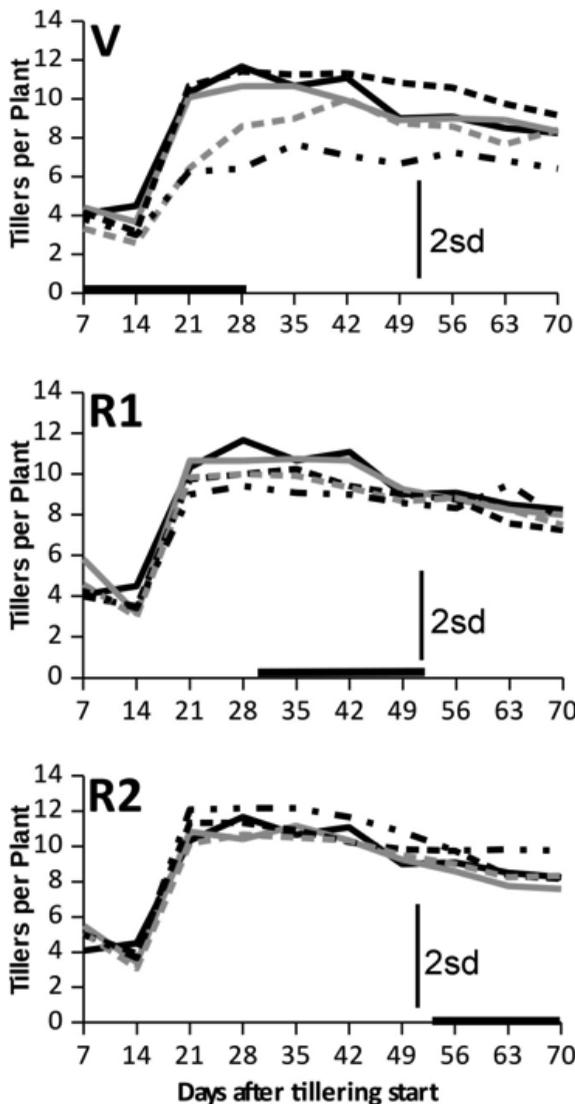


Figure 4 - Number of tillers per plant as function of distinct water stress levels imposed to different developmental stages of rice plants cv. BRSQuerencia. V = vegetative stage (from tillering start to panicle differentiation); R1 = reproductive 1 (from panicle differentiation to anthesis); R2 = reproductive 2 (from anthesis to ripening start). (—) Flood / (---) 10kPa / (- - -) 30 kPa / (- · - ·) 100V/60R kPa / (- · · · -) 200V/130R kPa.

The number of tillers per plant was also affected by the water stress, but only at the "V" stage (Figure 4), when water tensions of 100 and 200 kPa reduced tillering, respectively, in about 30 and 48%. Under 100 kPa, tillering was smoothly recovered after stress was removed, while for 200 kPa no recovery was observed in tillering after the stress was stopped (Figure 4). At the "V" stage, for the flooded check as well as for treatments until 30 kPa, only the usual tiller mortality by intraspecific competition was reported (Figure 4), being the same observed for the "R1" and "R2" stages. Considering the usual tiller mortality by competition, a maximum of 11-12 tillers was reported per plant, being reduced to 8-9 tillers per plant at the end of the cycle (Figure 4).

Leaf area (Figure 5) was about 200-380 cm² per plant (0.02-0.038 m² per plant) for all treatments until seven DAT (about 18 days after emergence - DAE), after this period it started growing fast and the maximum leaf area was observed about 28 DAT / 39 DAE, reaching about 0.18-0.25 m² plant⁻¹. Leaf area increase was halted for stress levels above 100 and 130 kPa, for "V" stage and above 130 kPa for "R1" stage (Figure 5); leaf area was slowly recovered after the stress was removed. For the "R2" stage no leaf area reduction was observed for any water stress level (Figure 5). For any treatment whose stress level did not cause leaf area restrictions, the leaf area curve was the usual, e.g., it slowly decreased after reaching a peak.

The shoot water content (Figure 6) was equivalent for all treatments in flooded, "V" and "R1" stages, being of about 63%; for "R2", mean water content in rice plants was 47%. Inside each developmental group ("V","R1","R2"), there was no association between rice shoot water content and the stress level to which plants were submitted.

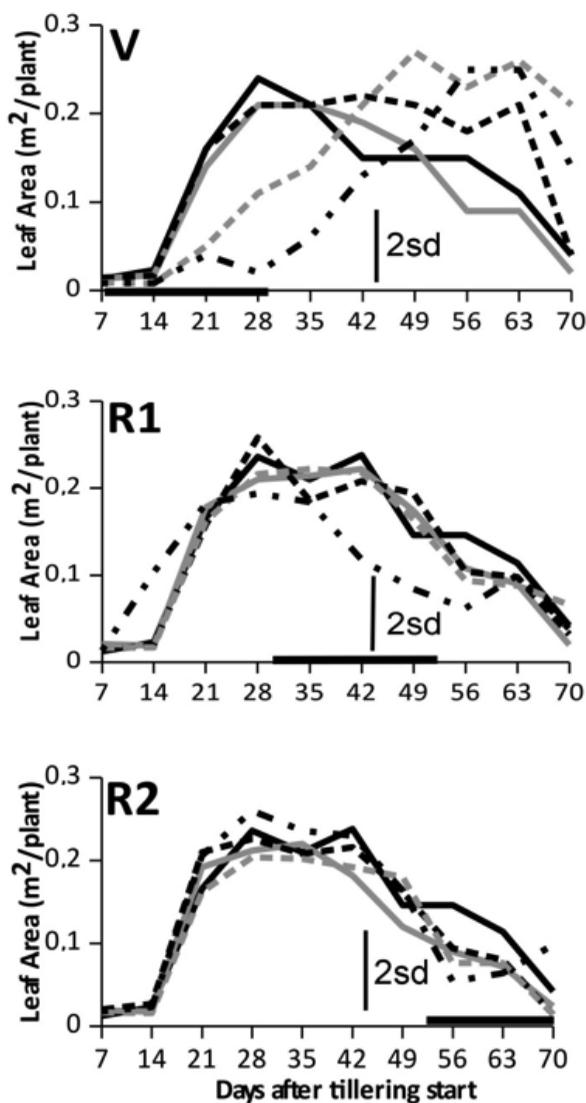


Figure 5 - Leaf area ($m^2\ plant^{-1}$) as function of distinct water stress levels imposed to different developmental stages of rice plants cv. BRSQuerencia. V = vegetative stage (from tillering start to panicle differentiation); R1 = reproductive 1 (from panicle differentiation to anthesis); R2 = reproductive 2 (from anthesis to ripening start). (—) Flood / (---) 10kPa / (· · ·) 30 kPa / (- · - ·) 100V/60R kPa / (- · - · -) 200V/130R kPa.

Shoot dry mass was divided into leaf and culm dry mass (Figure 7). Culm dry mass was of about $12\ g\ plant^{-1}$ for the flooded check, while it was about $8.52, 7.96$ and $11.3\ g\ plant^{-1}$, respectively for “V”, “R1” and “R2”. Water stress imposed at “R2” did not affect culm dry mass since rice plants had already reached its maximum growth. For “R1”, only the higher water stress (130 kPa) resulted in lower dry

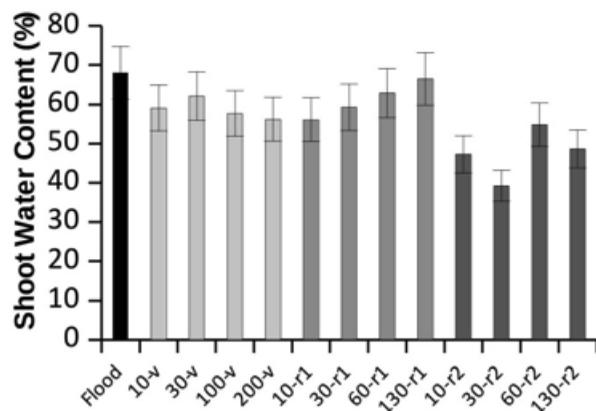


Figure 6 - Shoot water content as function of distinct water stress levels imposed to different developmental stages of rice plants cv. BRSQuerencia. V = vegetative stage (from tillering start to panicle differentiation); R1 = reproductive 1 (from panicle differentiation to anthesis); R2 = reproductive 2 (from anthesis to ripening start). Statistical differences among treatments, into each variable, were not significant.

mass ($8.59\ g\ plant^{-1}$ as mean of R110, R130 and R160, compared to $6.05\ g\ plant^{-1}$ for R1130) (Figure 7). Leaves dry mass (Figure 7) was about $3.83\ g\ plant^{-1}$ for “F” (flooded), “V”, and “R1”, and raised to about $5.82\ g\ plant^{-1}$ for treatments imposed in “R2”.

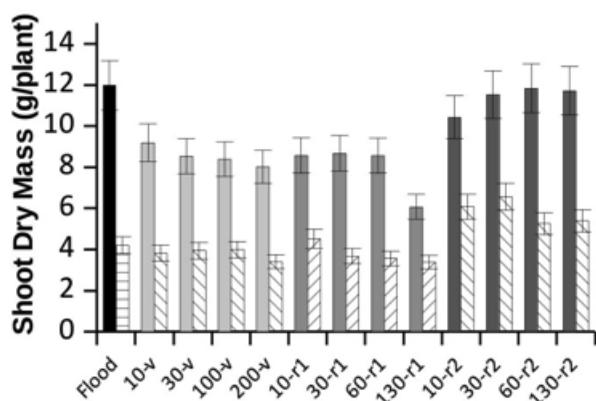


Figure 7 - Shoot dry mass of culms (■) and leaves (▨) as function of distinct water stress levels imposed to different developmental stages of rice plants cv. BRSQuerencia. V = vegetative stage (from tillering start to panicle differentiation); R1 = reproductive 1 (from panicle differentiation to anthesis); R2 = reproductive 2 (from anthesis to ripening start). Statistical differences among treatments, into each variable, were not significant.

Root volume (Figure 8) was about 48 cm³ plant⁻¹ for the flooded treatment, being superior to any of the irrigated treatments. For treatments applied at the “V” stage, root volume decreased from 31 to 15 cm³ plant⁻¹ when water stress applied at that stage was increased from 10 to 200 kPa (Figure 8), being the root volume decreased by 0.075 cm³ plant⁻¹ for each additional kPa of water stress. When the stress was applied at “R1” or “R2” there was no clear effect of water tension on root volume, averaging 27.5 and 31.6 cm³ plant⁻¹, respectively (Figure 8).

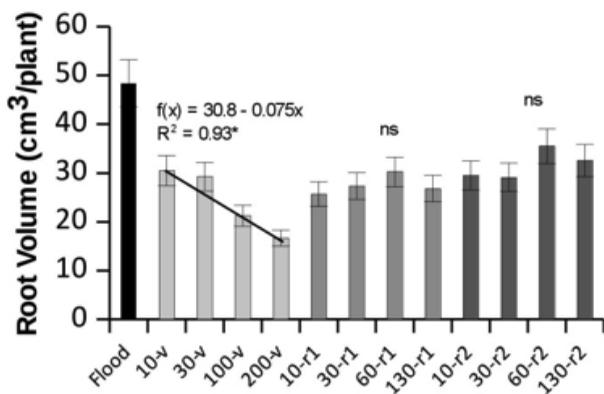


Figure 8 - Root volume (cm³ plant⁻¹) as function of distinct water stress levels imposed to different developmental stages of rice plants cv. BRSQuerencia. V = vegetative stage (from tillering start to panicle differentiation); R1 = reproductive 1 (from panicle differentiation to anthesis); R2 = reproductive 2 (from anthesis to ripening start).

DISCUSSION

Plant height was clearly affected when water stress was imposed at “V” and “R1” stages (from tillering start to anthesis) (Figure 3); while plants under stress at “V” were able to resume growth and completely restore the plant height after the stress was removed, those plants which underwent water stress at “R1” did not recover from the stress. These results are according to Gomes and Magalhães Jr. (2004) who reported that rice is able to recover from moderate water stress levels imposed at the vegetative stage, keeping its grain yield potential at harvest. One of the main issues of rice plants with smaller size in production fields, would probably be their reduced ability to

compete against weed species (Aldrich, 1984), but on the other side it would also reduce crop lodging risk (Gomes and Magalhães Jr., 2004).

Since tillering (Figure 4) was affected only when the water stress was imposed at “V”, with no recovery throughout the end of the cycle, it is possible to infer that the small number of tillers per plant under stress at “V” is minimally compensated by increased leaf area (Figure 5) which was formed after the stress imposed at “V” was withdrawn. Leaf area is not able to compensate the decrease on the number of grains, consequence of the reduction on the number of tillers. Zain *et al.* (2014) reported rice tillering mortality and reduced plant height under water stress, but these authors did not assess rice leaf area. Regarding overall plant growth as function of periods of stress, these authors also found that water stress periods lasting for a maximum of five days did not affect rice growth and development.

No water content changes were reported at the present study as function of the distinct water stress levels in soil, at any developmental stage; however, Biswas and Choudhuri (1984) reported water content decrease in rice plants as a function of drought stress. In fact, shoot water stress in rice plants may occur even when there is no drought and water is available, since rice roots have an inherent poor ability to conduct water, causing, in specific conditions, plant water deficit even when roots are into water (Miyamoto *et al.*, 2001). Thus, decreased rice shoot water content may depend mostly on climatic conditions (air humidity and temperature) than on the amount of excessive water present in soil. This represents another evidence that rice may be safely grown under sprinkler irrigation, with no harm to plant development, since irrigation is properly managed.

Regarding the developmental stages in which rice is most susceptible to water stresses, Blum (2011) reports that there is a big amount of experimental data which points for a general rule that plants are usually most severely affected by water stress at the reproductive stage, mainly near flowering (anthesis). When stress is imposed to rice between panicle initiation and anthesis, although a naturally slow rice plant growth is reported in this period, the damage in plant height and tillering

is not recovered and plant yield potential may be decreased (Biswas and Choudhuri, 1984).

At the present study, rice plant development was mostly affected by stresses imposed at the vegetative and reproductive stages until flowering. Our findings, however, are according to the reported by Blum (2011) and Biswas and Choudhuri (1984), since rice damage caused by stresses applied to the reproductive stage were not recovered after the stress was withdrawn.

When the water stress was imposed in "R1" and "R2", plants seemed to direct their reserves of energy to form roots (Figure 8) and try to escape severe water stress. In the "V" stage, the plant did not have enough reserves and root volume was decreased as the stress level increased. It is usual in Brazilian rice fields to leave rice go through a moderate water stress by the beginning of tillering, as a way to allow increasing in root volume. In the present study, under controlled environment, this was not observed; however, Biswas and Choudhuri (1984) reported that although some water stress did not increase rice development, it reflected in superior yield components at harvest. Our data supplies evidence that this management may not increase root volume or any other growth parameter for currently available early cycle rice varieties.

A relevant result is the much higher root volume at the constantly flooded check compared to the other treatments (Figure 8). Klumb *et al.* (2012) suggested that the demand of nutrients for rice grown under aerated soils may be higher than the required by flooded rice, due to a series of chemical reactions which could turn fertilizers most available for rice under flooding; thus, additional fertilizer could be needed for rice grown under sprinkler irrigation compared to flooding to keep equivalent plant

growth ability and grain yield potential. This, however, could be partially managed by a proper fertilizer formulation (Yang *et al.*, 2012).

Considering the plant height, tillering, leaf area and root volume, it is prudent to report that rice plants growth seem to be little affected by the absence of waterlogging, being possible to be grown under sprinkler irrigation in highlands since water is properly managed to avoid water stress in soil above 30 kPa throughout the cropping cycle.

FINAL CONSIDERATIONS / CONCLUSIONS

Rice plant height is reduced when the water stress is applied either at the vegetative or at the beginning of the reproductive stage (from panicle initiation to anthesis); in the first case, plants are able to later recover from the stress, but this is not true when stress was imposed at the beginning of the reproductive stage.

Tillering was affected only by water stress imposed at the vegetative stage, and plants did not recover after the stress was withdrawn.

Plant water content was not affected by the water stress, but shoot dry mass of culms was reduced when stress was applied between tillering start and anthesis.

Root volume did not increase as a function of water stress imposed at the vegetative stage.

Rice plants growth seem to be little affected by the absence of waterlogging, but there is the need to avoid water stress levels above 30 kPa throughout the cropping cycle.

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