



Categorization of the water status of rice inoculated with arbuscular mycorrhizae and with water deficit¹

Categorización del estado hídrico del arroz inoculado con micorrizas arbusculares y con déficit hídrico

Michel Ruiz-Sánchez², Juan Adriano Cabrera-Rodríguez², José M. Dell'Amico-Rodríguez²,
Yaumara Muñoz-Hernández³, Ricardo Aroca-Álvarez⁴, Juan M. Ruiz-Lozano⁴

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² National Institute of Agricultural Science (INCA9), Scientific and Technological Unit "Los Palacios", Gaveta Postal 1, San Jose de las Lajas, 37200, Mayabeque, Cuba. mich@inca.edu.cu (Corresponding Author: <http://orcid.org/0000-0002-7406-4715>); nani@inca.edu.cu (<http://orcid.org/0000-0002-0850-9050>); josemdellamico@gmail.com (<https://orcid.org/0000-0002-8196-2839>).

³ Municipal University Center "Los Palacios", Pinar del Río, Cuba. yaumara@upr.edu.cu (<https://orcid.org/0000-0001-9917-4796>).

⁴ Zaidín Experimental Station (CSIC), Department of Soil Microbiology and Symbiotic Systems. Professor Albareda n° 1, E-18008 Granada, Spain. raroca@eez.csic.es (<https://orcid.org/0000-0003-2402-2063>), juanmanuel.ruiz@eez.csic.es (<https://orcid.org/0000-0002-4473-5434>).

Abstract

Introduction. The water deficit negatively affects rice plants and limits their productivity. Arbuscular mycorrhizal symbiosis has been shown to improve rice productivity in drought conditions. **Objective.** To propose a new categorization for the state of water stress of rice plants inoculated (AM) or not with arbuscular mycorrhizal fungi (nonAM) and exposed to water deficit (D) during the vegetative phase. **Materials and methods.** The experiment was carried out under controlled greenhouse conditions during the years 2009 and 2010 at the Zaidín Experimental Station, Granada, Spain. The rice transplantation was carried out fourteen days after germination to pots with a 5 cm water sheet and at 30, 40, or 50 days after transplantation (DAT) they were subjected to water deficit during a period of 15 days, at which time the water sheet was restored. The control treatment was maintained throughout the cycle under flood conditions (ww). Evaluations were performed at 45, 55, 65 DAT and after recovery at 122 DAT. The harvest was carried out at 147 DAT. **Results.** The reduction in water supply demonstrated water stress in the plants, manifested by the decrease in the water potential of the leaves. Arbuscular mycorrhizal symbiosis always favored the water status of the plant. Four categories of water status of plants were proposed taking into account water potentials and agricultural yield: no stress (≥ -0.67 MPa); light stress (< -0.67 to -1.20 MPa); moderate stress (< -1.20 to -1.60 MPa), and severe stress (< -1.60 MPa). **Conclusion.** The categorization of stress due to the water deficit is a tool of high scientific value for the specific case of rice, since this plant has the capacity to adapt to tolerate the presence of a sheet of water throughout its biological cycle and is highly susceptible to water deficit.

Keywords: drought, agricultural yield, *Oryza sativa*.

Resumen

Introducción. El déficit hídrico afecta negativamente a las plantas de arroz y limita su productividad. Se ha demostrado que la simbiosis micorrízica arbuscular mejora la productividad del arroz en condiciones de sequía.



Objetivo. Proponer una nueva categorización para el estado de estrés hídrico de las plantas de arroz inoculadas (MA) o no con hongos micorrízicos arbusculares (noMA) y expuestas al déficit hídrico (D) durante la fase vegetativa. **Materiales y métodos.** El experimento se llevó a cabo bajo condiciones controladas de invernadero durante los años 2009 y 2010 en la Estación Experimental del Zaidín, Granada, España. El trasplante del arroz se realizó catorce días después de la germinación a macetas con una lámina de agua de 5 cm y a los 30, 40 o 50 días después del trasplante (DAT) se sometieron a déficit hídrico durante un período de quince días, momento en que se restableció la lámina de agua. El tratamiento de control se mantuvo durante todo el ciclo en condiciones de inundación (ww). Las evaluaciones se realizaron a los 45, 55, 65 DAT y después de la recuperación a 122 DAT. La cosecha se efectuó a los 147 DAT. **Resultados.** La reducción del suministro de agua evidenció estrés hídrico en las plantas, manifestado por la disminución del potencial hídrico de las hojas. La simbiosis micorrízica arbuscular siempre favoreció el estado hídrico de la planta. Se propusieron cuatro categorías de estado hídrico de plantas teniendo en cuenta los potenciales hídricos y el rendimiento agrícola: sin estrés ($\geq -0,67$ MPa); estrés ligero ($< -0,67$ a $-1,20$ MPa); estrés moderado ($< -1,20$ a $-1,60$ MPa) y estrés severo ($< -1,60$ MPa). **Conclusión.** La categorización del estrés debido al déficit hídrico es una herramienta de alto valor científico para el caso específico del arroz, ya que esta planta tiene la capacidad de adaptación para tolerar la presencia de una lámina de agua durante todo su ciclo biológico y es altamente susceptible al déficit hídrico.

Palabras clave: sequía, rendimiento agrícola, *Oryza sativa*.

Introduction

The ongoing climate change is characterized by increased temperatures and altered precipitation patterns. In addition, there has been an increase in both the frequency and intensity of extreme climatic events such as drought (Field et al., 2014; Brunner et al., 2015). Moreover, the global climate change is contributing to spread the water deficit problems to regions where drought was negligible in the past (Trenberth et al., 2014). Water deficit is considered the most important abiotic factor limiting plant growth and yield in many areas (Bray, 2004; Trenberth et al., 2014). The severity of drought depends on many different factors including evaporative demands, rainfall levels and moisture-storing capacity of soils (Farooq et al., 2014). Drought stress has now become a severe threat to ensure food security in the developing world.

In plants, drought induces biochemical, physiological, morphological, and molecular changes. Thus, most plant processes are affected directly or indirectly by the water limitation (Ruiz-Lozano et al., 2012). Different plant species vary in their sensitivity and response to water deficit, although it is assumed that all plants have encoded capability for stress perception, signaling and response (Bohnert et al., 1995; Golldack et al., 2014). Plants have developed several mechanisms to cope with drought stress such as morphological adaptations, osmotic adjustment, optimization of water resources, improvement of antioxidant system, reduction of growth and photosynthesis rate and stomatal closure, all aimed to optimize water use (Farooq et al., 2009; Osakabe et al., 2014; Ruiz-Lozano et al., 2012; Shinozaki & Yamaguchi-Shinozaki, 2006).

Rice plays a major role as a staple food, supporting more than three billion people and comprising 50 to 80 % of their daily calorie intake (Khush, 2003). The demand for rice production is still rising because of the continuous increase in world population. The world population is predicted to reach approximately 8 billion by 2030, and there is therefore a need to further increase rice production by 40 % in the next 20 years (Bernier et al., 2008). However, drought stress severely impairs its production. Worldwide, drought affects approximately 23 million hectares of rainfed rice (Serraj et al., 2011). This cereal requires larger amount of water throughout its life cycle as compared to other crops. Hence, water stress causes severe threat to rice production, with drought being a major challenge

limiting rice production. This cereal requires larger amount of water throughout its life cycle as compared to other crops. Hence, water stress causes severe threat to rice production, with drought being a major challenge limiting rice production. It has been estimated that more than 50 % of the world's arable land will be affected by drought in the year 2050 (Singhal et al., 2016). The increase in drought severity coupled with lack of high-yielding genotypes that are suitable for cultivation under drought conditions are the most limiting factors responsible for low production of rice. Rice cultivation is seasonal due to a lack of appropriate rice cultivars and techniques (Oladosu et al., 2019).

The rice crop has the peculiarity to be a semi-aquatic plant; however, it is traditionally cultivated in continuous flooding conditions for most of its development cycle (Hattori et al., 2011). Although rice plants have few adaptations for limited water supply conditions and it is sensitive to drought (Kamoshita et al., 2008), the exposure of the crop to water deficit in the vegetative phase has favored the increase of yield, as reported by different researchers (García et al., 2009; Polón, 2007).

Crop responses to drought stress and its tolerance level can be measured by monitoring different physiological changes during drought period. Taking into account the current and future forecast of water scarcity and the importance of rice crop, it is necessary to explore the potential of the application of water deficit periods in order to keep or improve rice production, as well as, knowing the water potential of rice plants when expresses its maximum yield potential. Under conditions of water deficit, plants tend to control root water uptake and/or tissue water status by adjusting root hydraulic conductivity and osmotic adjustment to maintain a gradient of water potential favorable to plant entrance into the root (Bárcana et al., 2012), at the same time that it favors the leaf water potential of plant.

The leaf water potential allows measuring the plant water status during the day. It is a method which enables the measurement of a short-term hydric response (for example on an hourly basis) of plant in reaction to a change in the root water absorption and the leaf transpiration (interaction soil water content x climate x leaf transpiration x cultivar). The leaf water potential is a variable of great reliability and used to describe the water status of a plant (Azcón-Bieto & Talón, 2000; Taiz & Zeiger, 2006), this variable is taken as a reference for all the agricultural cultivations of interest, including rice.

With the purpose of estimating the hydric state of the plants, other physiological parameters indicative of it have been carried out, such as perspiration and stomatal conductance, CO₂ assimilation, respiration, cell growth and cell division of cell synthesis, hormones and ethylene, nitrogen metabolism, enzyme levels and mechanisms underlying responses to water stress (Hsiao, 1973). It is important to note that the Hsiao (1973) proposed moderate water deficit category included a very broad range ranging from -5 bar to -12 or -15 bar (= -0.5 MPa to -1.2 or -1.5 MPa), and in this work it was possible to determine and establish another range of water stress. In spite of the above, from the 1970s to the present, several authors have used the categorization proposed by this author to compare their results, which was recently used in research on rice by García et al. (2009).

Studies by Carbonneau (1998) suggest threshold values or critical levels of water potential in the case of vine cultivation, based on the result of 20 or more years of observations in many vineyards of different cultivars. This reliable and validated tool is conducive to appropriate sampling at the plot level (Deloire & Heyns, 2011). It is considered critical level in the plants at physiological level, all those abiotic factors that affect their growth and development, which by excess or by default cause the decrease of their yield. There are critical levels of temperature, light, oxygen, carbon dioxide, soil, availability of water and nutrients, which affect physiological processes such as; photosynthesis, respiration, perspiration, absorption of water and nutrients, among others. (Azcón-Bieto & Talón, 2000; Degrovani et al., 2010; Taiz & Zeiger, 2006).

Several studies have described how mycorrhizal symbiosis favors the water status of various plant species in the presence of a water deficiency in the soil (Aroca et al., 2008); that these microorganisms contribute to increase relative water content, transpiration rate, and CO₂ exchange (Bárcana et al., 2015; Ruiz-Sánchez et al., 2010; Ruiz-Lozano et al., 2012; Ruiz-Sánchez et al., 2015), thus ensuring a better water status of plant.

The objective of the present work was to propose a new categorization for the state of water stress of rice plants inoculated (AM) or not with arbuscular mycorrhizal fungi (non AM) and exposed to water deficit (D) during the vegetative phase.

Materials and methods

Experimental design

The experiment was carried out under controlled greenhouse conditions in 2009 and 2010, at the Zaidin Experimental Station, Consejo Superior de Investigaciones Científicas (CSIC), Granada, Spain, with rice plants (*Oryza sativa*) cv. INCA LP-5 (Pérez et al., 2018). The rice plants were exposed to water stress at three different times in the vegetative phase, following a completely randomized experimental design, with five replications.

Treatments

- T1. Plants condition under well-watered (ww).
- T2. Plants under water stress or drought (D) at 30 days after the transplant (DAT).
- T3. Plants condition under well-watered (ww).
- T4. Plants under water stress or drought (D) at 40 days after the transplant (DAT).
- T5. Plants condition under well-watered (ww).
- T6. Plants under water stress or drought (D) at 50 days after the transplant (DAT).

Soil and biological materials

A seedbed of rice was set in plastic trays of 0.40 x 0.80 x 0.08 m with sterile sand. Fourteen days old rice seedlings were transplanted, single plant to each of the 1 kg pots (0.18 m high and 0.13 m diameter) containing a substrate composed of sand (granulometry <1 mm) and soil (granulometry < 5 mm) in a 1:1 ratio (v:v), which was previously sterilized. The sand was sterilized at 120 °C for 20 min in a Selecta autoclave; model PRESOCCLAVE-II 75 L, while the soil was sterilized by steaming at 95-100 °C, for 60 min on three consecutive days. The soil (collected from the Zaidin Experimental Station, Granada, Spain) had a pH of 8.1 (water); 1.81 % organic matter, nutrient concentrations (mg kg⁻¹): N, 2.5; P, 6.2 (NaHCO extractable P); K, 132.0. The soil texture was made up of 35.8 % sand, 43.6 % silt, and 20.5 % clay.

Mycorrhizal inoculum was bulked in an open-pot culture of *Zea mays* L. and consisted of soil, spores, mycelia and infected root fragments. The AM species was *Rhizoglyphus intrarradices* (Schenck and Smith), isolate EEZ 01 (56 spores g⁻¹ of inoculum). Prior to transplantation, plants were inoculated with *Rhizoglyphus intrarradices*, at a rate of 5 g of inoculum per pot, deposited under the plant. 50 % of them were inoculated, while the other 50 % remained non-inoculated. These no-inoculated control treatments (nonAM) received a 10 ml aliquot of filtrate (<20 µm) of the AM inoculates to provide a general microbial population free of AM propagules.

Growing conditions

The pots were placed in the greenhouse where the seedbed was established and remained throughout the experiment with temperatures of 26 °C and 22 °C (day / night, respectively); relative humidity between 50-70; photoperiod of 16 hours of light and 8 hours of darkness and photosynthetically active radiation of 850 µmol m⁻² s⁻¹, measured with a

portable LIQUOR (Lincoln, NE, USA, model LI-188B), following an experimental design completely randomized, with five repetitions, for which 25 pots were used per treatment.

The irrigation consisted of maintaining a 5 cm sheet of water daily on the surface of the substrate in all the treatments until the different irrigation management was applied. The irrigation treatments consisted in the suspension of the water sheet at 30, 40, and 50 days after the transplant (DAT) for a period of 15 days and a treatment without suspension of the water sheet (Control). After the period without water sheet, the water sheet (5 cm) was replaced every other day until 15 days before harvest, which was the same at 147 DAT.

The total nutrient application, corresponding to 0.123 g of N, 0.050 g of P_2O_5 , and 0.059 g of K_2O per pot, was performed at 20 DAT (30 % of total dose), 35 DAT (40 % of total dose), and 60 DAT (30 % of total dose). Urea (46 % N), triple superphosphate (46 % P_2O_5) and potassium chloride (60 % K_2O) were used as fertilizers.

Measurements

Soil moisture

Soil moisture was determined after the suspension period of the water sheet (at 45, 55, and 65 DAT), with a Theta Probe Soil Moisture Sensor - ML2x and was expressed in cm^3 of water per cm^3 of substrate.

Mycorrhizal colonization

The percentage of mycorrhizal root infection was estimated by visual observation of fungal colonization after clearing washed roots in 10 % KOH and staining with 0.05 % Trypan blue in lactic acid (v/v), according to Phillips & Hayman (1970). Quantification of the root colonization was performed according to the grid-line intersect method (Giovannetti & Mosse, 1980). Five replicates per treatment were used.

Water potential

Five plants per treatment were taken 15 days after starting the water stress (45, 55, and 65 DAT) and at 122 DAT (25 days before harvest), for leaf water potential (Ψ_h), with the purpose of measuring the values of leaf water potential. The leaf water potential (Ψ_h) was determined by the psychrometric method, two hours after the start of the daytime period (between 9:00 a.m. and 10:00 a.m.), using a microvolt meter HR-33T connected to a psychrometric chamber C52 (Wescor Inc, Logan, UT, USA), as described by Porcel & Ruiz-Lozano (2004). Ten discs (0.005 m diameter) were taken per treatment, that is two per plant from the central part of the leaves of the plant and placed in the chamber for 15 min to stabilize the temperature and water vapor of the disc before read Ψ_h , which was expressed in MPa.

Agricultural yield

Agricultural yield per plant ($g\ plant^{-1}$) was evaluated at the time of harvest (147 DAT) when 85 % of the spikes in the plant turned yellow (International Rice Research Institute, 2013). The production of the five plants was harvested independently and the production of the grains per plant was weighed. The relative agricultural yield (RR %) was estimated, dividing the yield values obtained between the maximum yield value and then multiplied by 100. The values of RR % were used to determine the critical levels of Ψ_h . The critical levels of Ψ_h were determined following the mathematical procedure proposed by Cate & Nelson (1971), considering first all the studied treatments and then subdividing the populations until reaching the stress categories.

Statistic analysis

The data were subjected to analysis of simple variance (ANOVA), plants inoculated with arbuscular mycorrhizal fungi (AMF), exposed or not to water stress, from which significant interaction was found between the factors under study, the data were submitted to the Test Duncan's Multiple Range (Duncan, 1955). After the recovery period (at 122 DAT), the data are processed following the same procedure as after each period of water stress (D), with the exception that the values are compared against a single control without water stress (ww).

Results

No mycorrhizal colonization was observed in plants not provided with AM inoculum due to the sterilization to which the substrate was subjected. The mycorrhizal colonization in rice plants that remained under flooding conditions (ww) and in those that were exposed to drought stress (D), while in the inoculated plants an increase of this variable was found over time (Figure 1).

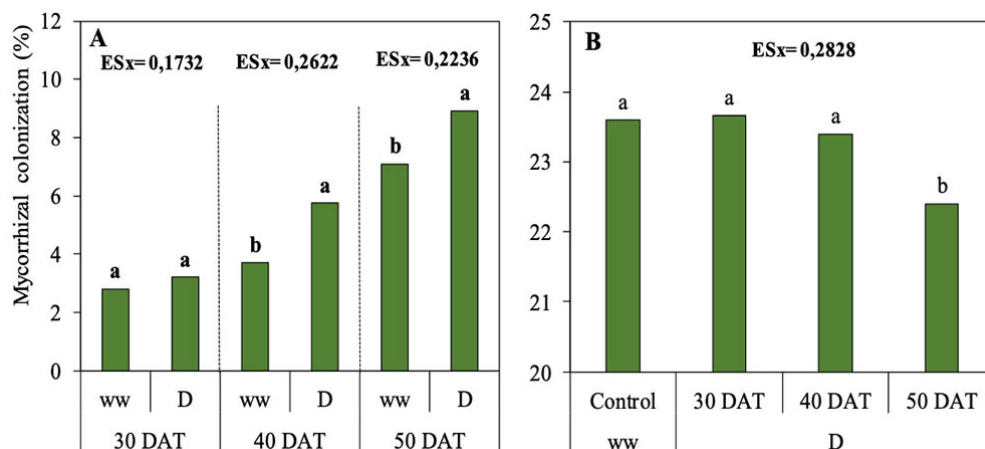


Figure 1. Mycorrhizal colonization, 15 days after starting the reduction of water supply (A) and 25 days before harvest, at 122 days after transplantation (DAT) (B) in rice plants (*O. sativa*) inoculated with *R. intrarradices* (AM) and non-inoculated (nonAM), cultivated with a sheet of water (ww) or exposed to a reduction of water supply (D) at 30, 40, and 50 DAT, respectively, at the Zaidin Experimental Station, Consejo Superior de Investigaciones Científicas (CSIC), Granada, Spain. 2009-2010.

Means followed by the same letter are not significantly different ($p>0.05$) as determined by Duncan's multiple range test ($n=5$).

Figura 1. Colonización micorrízica, 15 días después de comenzar la reducción del suministro de agua (A) y 25 días antes de la cosecha, a 122 días después del trasplante (DAT) (B) en plantas de arroz (*O. sativa*) inoculadas con *R. intrarradices* (MA) y no inoculadas (noMA), cultivadas con lámina de agua (ww) o expuestas a una reducción del suministro de agua (D) a los 30, 40 y 50 DDT, respectivamente, en la Estación Experimental Zaidín, Consejo Superior de Investigaciones Científicas (CSIC), Granada, España. 2009-2010.

Las medias seguidas de la misma letra no son significativamente diferentes ($p>0,05$) según lo determinado por la prueba de rango múltiple de Duncan ($n=5$).

At 45 DAT no significant differences were observed between the plants maintained under flooding conditions (ww) and those exposed to water stress (D); however, at 55 and 65 DAT higher colonization values were observed in treatments that were exposed to water stress. In the recovery at 122 DAT (Figure 1B), the mycorrhizal colonization in the control plants and those that were exposed to water stress at 30 and 40 DAT were similar and in the first two treatments there was more colonization than when the plants were stressed at 50 DAT.

The above data showed that the largest increase in colonization, between the first evaluation and after recovery was found in stressed plants. In this sense, in stressed plants at 30 DAT the colonization increase was 20 %, when the stress was imposed at 40 DAT the increase was 17.5 % and finally, the stress at 50 DAT increased the colonization by 13 %, compared to the post-recovery indicator stress assessment (122 DAT).

The results of agricultural yield in rice plants exposed to a reduction of water supply for a period of 15 days during the vegetative phase (30, 40 and 50 DAT) and inoculated or not with *R. intrarradices* are shown in the Figure 2. The lowest yield was found in plants that were not exposed to water deficit and were not inoculated. This parameter decreased when the water stress was imposed at 50 DAT, with respect to the treatments that were exposed to this condition at 30 and 40 DAT, although the yield was higher than in the control treatment without water stress (ww).

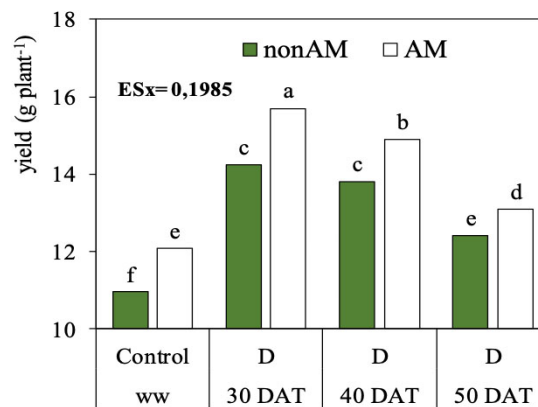


Figure 2. Agricultural yield at the time of harvest (147 DAT) in rice plants (*O. sativa*) inoculated with *R. intrarradices* (AM) and non-inoculated (nonAM), cultivated with sheet of water (ww) or exposed to a reduction of water supply (D) at 30, 40, and 50 DAT, respectively, at the Zaidin Experimental Station, Consejo Superior de Investigaciones Científicas (CSIC), Granada, Spain. 2009-2010.

Means followed by the same letter are not significantly different ($p>0.05$) as determined by Duncan's multiple range test ($n=5$). DAT: days after transplantation.

Figura 2. Rendimiento agrícola en el momento de la cosecha (147 DAT) en plantas de arroz (*O. sativa*) inoculadas con *R. intrarradices* (MA) y no inoculadas (noMA), cultivadas con lámina de agua (ww) o expuestas a una reducción del suministro de agua (D) a los 30, 40 y 50 DDT, respectivamente, en la Estación Experimental Zaidín, Consejo Superior de Investigaciones Científicas (CSIC), Granada, España, 2009-2010.

Las medias seguidas de la misma letra no son significativamente diferentes ($p>0,05$) según lo determinado por la prueba de rango múltiple de Duncan ($n=5$). DAT: días después del trasplante.

The values of the water potential of the leaf (Ψ_h) are shown in Figure 3. The Ψ_h was higher in the plants that did not suffer a reduction of the water supply (ww) in the three moments (30, 40, and 50 DDT). The treatment with the lowest water content in the leaves was that which was exposed to water stress at 50 DDT (Figure 3 A). However, always the inoculation treatments with AM showed the highest values of Ψ_h with respect to the plants without AM.

The period of 15 days of water deficit in the soil (substrate) caused a water stress in the plant, which increased as the water deficit was imposed later, this was reflected in a decrease of Ψ_h (Figure 3 A); furthermore, under such conditions of water deficit, the inoculation caused Ψ_h values always higher than those found in non-inoculated plants. After recovery (122 DAT), values greater than Ψ_h in plants inoculated without stress and stressed at 30 DAT compared to the control treatments and those exposed to water stress at 40 and 50 DAT (Figure 3 B).

The results so far analyzed indicate that the Ψ_h reflected the water status of the plants exposed or not to water stress, which showed the pertinence of determining the critical levels or values of Ψ_h for rice related to relative yield (RR %). Since there is still no categorization of specific Ψ_h in rice plants exposed to this condition. Figure 4 shows the dispersion of values between Ψ_h and RR % found in the different conditions evaluated.

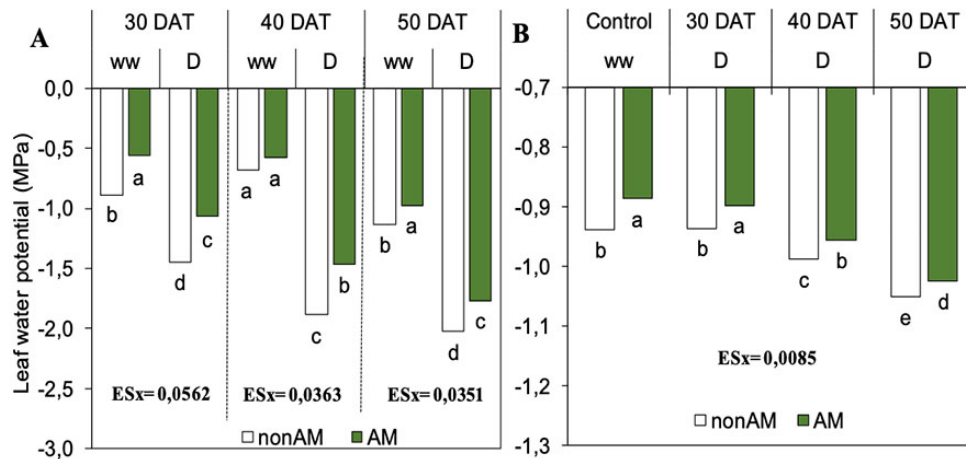


Figure 3. Leaf water potential, 15 days after starting the reduction of water supply (A) and 25 days before harvest (B), in rice plants (*Oryza sativa*) inoculated with *R. intrarradices* (AM) and non-inoculated (nonAM), cultivated with water sheet (ww) or exposed to a reduction of water supply (D) at 30, 40 and 50 DAT, respectively, at the Zaidín Experimental Station, Granada, Spain, 2009-2010.

Means followed by the same letter are not significantly different ($p>0.05$) as determined by Duncan's multiple range test ($n=5$).

Figura 3. Potencial hídrico de las hojas, 15 días después de comenzar la reducción del suministro de agua (A) y 25 días antes de la cosecha (B), en plantas de arroz (*Oryza sativa*) inoculadas con *R. intrarradices* (MA) y no inoculadas (noMA, cultivadas con lámina de agua (ww) o expuestas a una reducción del suministro de agua (D) a los 30, 40 y 50 DDT, respectivamente, en la estación experimental de Zaidín, Granada, España, 2009-2010.

Las medias seguidas de la misma letra no son significativamente diferentes ($p>0.05$) según lo determinado por la prueba de rango múltiple de Duncan ($n=5$). DAT: días después del trasplante.

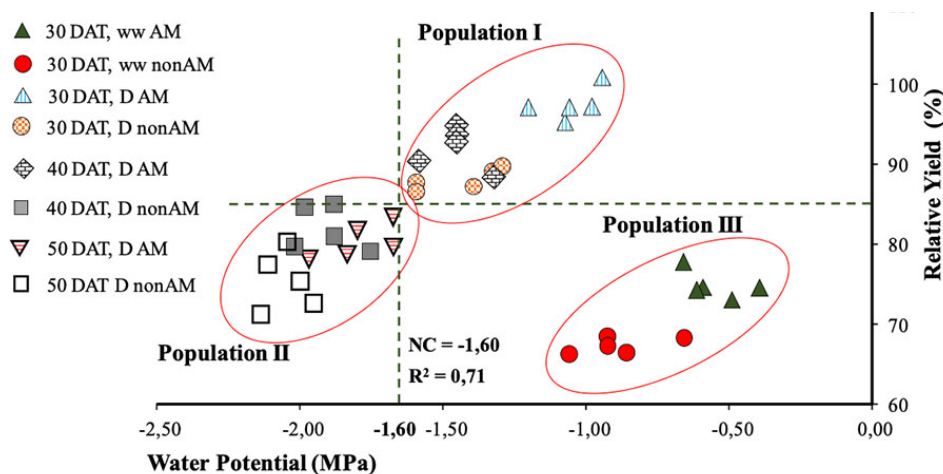


Figure 4. Critical level of foliar water potential (Ψ_h) determined 15 days after each period of water supply reduction, represented in a scatter diagram of the relationship between Ψ_h and the relative yield of rice plants (*Oryza sativa*) inoculated with *R. intrarradices* (AM) and non-inoculated (nonAM), cultivated with water sheet (ww) or exposed to a reduction of water supply (D) at 30, 40 and 50 DAT, respectively. The maximum yield with which the relative yield was calculated was $16.24 \text{ g plant}^{-1}$, at the Zaidín Experimental Station, Granada, Spain, 2009-2010.

Figura 4. Nivel crítico de potencial hídrico foliar (Ψ_h) determinado 15 días después de cada período de reducción del suministro de agua, representado en un diagrama de dispersión de la relación entre Ψ_h y el rendimiento relativo de las plantas de arroz (*Oryza sativa*) inoculadas con *R. intrarradices* (MA) y no inoculados (noMA), cultivadas con lámina de agua (ww) o expuestas a una reducción del suministro de agua (D) a los 30, 40 y 50 DDT, respectivamente. El rendimiento máximo con el que se calculó el rendimiento relativo fue de $16.24 \text{ g planta}^{-1}$, en la Estación Experimental Zaidín, Granada, España, 2009-2010.

Three well-defined populations were obtained, one in the upper right quadrant, one in the lower left quadrant and the other in the lower right quadrant. The first population was characterized by presenting values of RR % between 85 and 100 %, with a critical level of water potential up to -1.60 MPa, corresponding to plants not inoculated and inoculated with AM that were stressed at 30 DAT and those inoculated with AM and exposed to water stress at 40 DAT.

The second population with a maximum RR of 85 % and a critical water potential level higher than -1.60 MPa, corresponded to non-inoculated treatments exposed to water stress at 40 DAT and not inoculated and inoculated treatments exposed to water stress at 50 DAT. The third population corresponded to not inoculated and inoculated treatments which were never exposed to a stress situation.

The value of RR % that corresponds to the critical level indicates that the population of the upper right quadrant can be divided again. This second partition (Population I) was characterized by presenting a RR % of 95 %, associated to the critical level of Ψ_h -1.20 MPa and corresponded to the treatments exposed to water stress at 30 and 40 DAT, but inoculated with AMF and not inoculated (Figure 5).

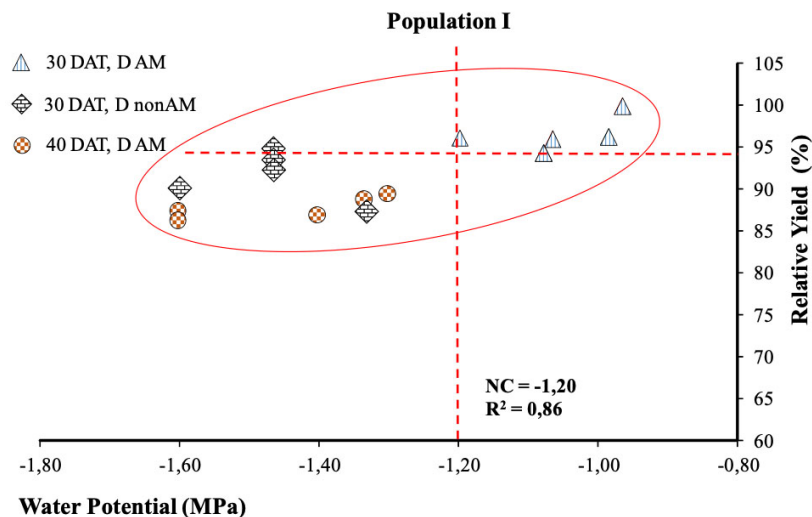


Figure 5. Critical level of leaf water potential (Ψ_h) determined 15 days after each period of reduction of water supply, represented in a dispersion diagram of the relationship between Ψ_h and the relative yield of rice plants (*Oryza sativa*) inoculated with *R. intrarradices* (AM) and non-inoculated (nonAM), cultivated with to a reduction of water supply (D) at 30 and 40 DAT, respectively. The maximum yield at which the relative yield was calculated was 16.24 g plant⁻¹, at the Zaidín Experimental Station, Granada, Spain, 2009-2010.

DAT: days after transplantation.

Figura 5. Nivel crítico de potencial hídrico foliar (Ψ_h) determinado 15 días después de cada período de reducción del suministro de agua, representado en un diagrama de dispersión de la relación entre Ψ_h y el rendimiento relativo de las plantas de arroz (*Oryza sativa*) inoculadas con *R. intrarradices* (MA) y no inoculados (noMA), cultivadas con reducción del suministro de agua (D) a los 30 y 40 DDT, respectivamente. El rendimiento máximo al que se calculó el rendimiento relativo fue de 16,24 g planta⁻¹, en la Estación Experimental Zaidín, Granada, España, 2009-2010.

DAT: días después del trasplante.

Finally, the third population formed by witness treatments, which were not subjected to water stress conditions, could be divided into two subpopulations, with a new critical level of Ψ_h of -0.67 MPa (Figure 6).

The analysis developed with the results allowed to establish a categorization of the degree of stress in which the rice plants were found from Ψ_h and RR % (Table 1).

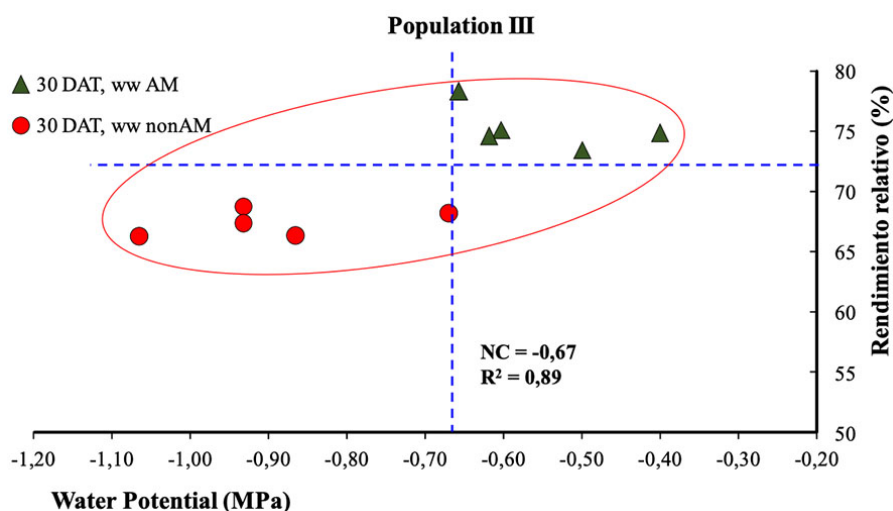


Figure 6. Critical level of leaf water potential (Ψ_h) determined 15 days after each period of water supply reduction, represented in a scatter diagram of the relationship between Ψ_h and the relative yield of rice plants (*O. sativa*) inoculated with *R. intrarradices* (AM) and non-inoculated (nonAM), cultivated under well-watered (ww) conditions, at 30 DAT. The maximum yield at which the relative yield was calculated was 16.24 g plant⁻¹, at the Zaidin Experimental Station, Consejo Superior de Investigaciones Científicas (CSIC), Granada, Spain, 2009-2010.

Figura 6. Nivel crítico de potencial hídrico foliar (Ψ_h) determinado 15 días después de cada período de reducción del suministro de agua, representado en un diagrama de dispersión de la relación entre Ψ_h y el rendimiento relativo de las plantas de arroz (*O. sativa*) inoculadas con *R. intrarradices* (MA) y no inoculados (noMA), cultivados en condiciones bien regadas (ww), a 30 DAT. El rendimiento máximo al que se calculó el rendimiento relativo fue de 16,24 g planta⁻¹, en la Estación Experimental Zaidín, Consejo Superior de Investigaciones Científicas (CSIC), Granada, España, 2009-2010.

Table 1. Categorization of water deficit stress in rice plants (*O. sativa*) as a function of leaf water potential (Ψ_h) (measured between 9:00 am and 10:00 am) and relative agricultural yield (RR), at the Zaidin Experimental Station, Consejo Superior de Investigaciones Científicas (CSIC), Granada, Spain, 2009-2010.

Cuadro 1. Categorización del estrés por déficit hídrico en plantas de arroz (*O. sativa*) en función del potencial hídrico foliar (Ψ_h) (medido entre las 9:00 a.m. y las 10:00 a.m.) y el rendimiento agrícola relativo (RR), en la Estación Experimental Zaidín, Consejo Superior de Investigaciones Científicas (CSIC), Granada, España, 2009-2010.

Stress category	Ranges of the Ψ_h (MPa)
No stress	≥ -0.67
Light stress	< -0.67 a -1.20
Moderate stress	< -1.20 a -1.60
Severe stress	< -1.60

Discussion

The values of mycorrhizal colonization reaffirmed the results obtained by other researchers, who found low colonization levels working with the same plant species and under flooding conditions (Vallino et al., 2009; 2014). Despite of the low levels colonization of AM root, AM symbiosis had an influence on Ψ_h and agricultural yield. The most probable cause that could motivate the low levels of mycorrhizal colonization is the anaerobiosis

condition and the production of ethylene in the medium. The anaerobiosis condition due to the existence of the water sheet, increases the ethylene concentration in the medium (Malik et al., 2003), compound which at high concentrations inhibits hyphal growth (Ishii et al., 1996).

Exposing the rice plant to a reduction of water supply for a period of 15 days at some moments in the vegetative phase guaranteed the increase in agricultural yield. The increases in the development of plants inoculated with arbuscular mycorrhizal fungi (AMF) was also reported by other researchers working on crops such as wheat (Ma et al., 2008), maize (Li et al., 2011), the latter under rainfed conditions and crop rotation with other crops.

The best hydric state of the plant is associated with the increase in Ψ_h , while it can be related to mycorrhizal inoculation, from possible hyphal growth, combined with the ability of the fungus to absorb water under low Ψ_h conditions (Augé, 2004; Lehto and Zwiazek, 2011). The increase in Ψ_h in the inoculated plants, led to greater growth and development of the plant, from the improvement of the Ψ_h gradient, which had to facilitate the flow of water to the elongating cells.

It seems that growth hormone exudates in the AMF-plant interaction (Pozo et al., 2015), stimulated greater growth and development observed through agricultural yield. In addition to this, greater efficiency in stomatic control, ways that the plant uses as an adaptation mechanism, to reduce water losses (Damour et al., 2010). Ruiz-Sánchez et al. (2010; 2011) reported similar results in rice plants exposed to water stress for a period of 15 days in the vegetative phase.

The results of this investigation corroborated the findings of other authors, as regards the increase in rice yield due to the effect of AM inoculation (Maiti et al., 2011, 2012; Rivera et al., 2007), intentional water stress (García et al., 2009; Polón & Castro 1999). García et al. (2009) subjected a rice cultivar to water stress in the vegetative phase (50 and 57 days after sowing, in the dry and rainy seasons, respectively) under semi-controlled conditions and found that plants with water stress showed an increase in agricultural yield, which ranged between 30 % and 43 % in the dry and rainy season, respectively.

At 40 DAT and 50 DAT, the plant could be at the beginning and development of the reproductive phase, so the plant metabolism may be being reserved for the change of primordium and flowering, processes which could be affected to a greater extent by the imposition of the reduction of the water supply, with respect to the 30 DAT.

A water deficit imposed on the rice plant in the final period of the vegetative phase (50 DAT, leads to severe water stress, which was evidenced through the values of water potential (<-160 MPa), due to foliar development reached by the plant, which consumes and exchanges a greater amount of water for evapotranspiration. It is possible that this water consumption is very related to the process of translocation of the leaves towards internodes in its first stage and also for the cellular elongation that should occur in the reproductive phase for the emission of the flower spike in the crop, therefore, it causes a decrease of the foliar water potential superior to the treatment with water deficit at 40 DAT and 30 DAT, respectively.

It is important to mention that at this time of evaluation (122 DAT, 25 days before harvest), the plants were in a process characterized by a greater demand for water for metabolite translocation and grain filling (García et al., 2010), as well as by senescence, an aspect that diminishes photosynthesis (Lim et al., 2007) and causes biochemical changes in the plant in order to complete its biological cycle.

The recovery of the plants to the water deficit (122 DAT) indicated the direct participation of the AM in the mechanisms of signaling and adaptation of the plants to the state of stress and recovery of the same. All of the above indicated that the behavior found for Ψ_h was influenced by the AM inoculation, since somehow the water absorption capacity of the plants and presumably the hydraulic conductivity of the root were increased, which was also corroborated in other studies (Dell'Amico et al., 2002; Smith et al., 2010), with different wheat genotypes (Silva-Robledo et al., 2007) and maize (Bárzana et al., 2012).

After the recovery period, according to Ψ_h values, it can be demonstrated the recovery capacity of the plants exposed to water stress, both in the AM as in the non AM plants treatments. In this respect, Polón (2007) reported

that rice has the capacity to recover from drought, even when water stress imposed can be considered as severe by its duration and intensity. If the water stress is severe, García et al. (2010) indicates that it decreases the yield and can cause the death of the plant. Both criteria suggest that there is no clear picture of how far and how long a rice plant can tolerate drought as an effect of water stress.

To the extent that water stress became more belatedly, it corresponded with the limitation of the growth and development of the rice plant, which could be attributed to a decrease of the entrance of CO₂ in the cells and a low efficiency in the use of the light, at the same time could cause the inactivation of the photosynthetic system and therefore, the plants did not produce sufficient assimilates that contributed to the cellular division of the meristematic tissue for the growth of the plant (Mostajeran and Rahimi-Eichi, 2009; Zubarer et al., 2007), and its accumulation for grain filling (García et al., 2010), while increasing ethylene production under these conditions and thus the senescence process (Malik et al., 2003).

The Ψ_h ranges presented were comparable to those proposed for all plants by Hsiao (1973), a proposal that was made from an analysis of published results on the effect of water stress on plants. However, this author defined three degrees of stress to categorize the water status of a plant, taking into account the values of Ψ_h and the relative water content (RWC). RWC is the water content (on a percentage basis) relative to the water content of the same tissue at full turgor (after floating on water to “constant” weight): Light water deficit: When Ψ_h is greater than -5 bar or when the RWC is reduced by 8-10 %. Moderate water deficit: When the Ψ_h is between -5 bar to -12 or -15 bar or when the RWC decreases between 10 and 20 %. Severe water deficit: When Ψ_h is less than -15 bar or when RWC decreases by more than 20 %.

Other studies related to leaf water potential and irrigation in vine plants (Carbonneau, 1998) proposed five categories of water stress for this crop, which included severe stress at potential less than 0.8 MPa. On the other hand, Deloire & Heyns (2011), Ojeda et al. (2002) and, Williams & Araujo (2002), proposed four categories and found that above 0.9 MPa, vegetative growth, photosynthesis and grape ripening were inhibited. These works did not apply a mathematical procedure that will substantiate the levels of proposed water potential.

Conclusions

The rice plants responded differently to water deficiency depending on the time of its imposition and plant phenological status. The arbuscular mycorrhizal symbiosis favored the hydric state of the plant when they are subjected to water deficit, which is evidenced by the water potential and the increase in yield, but this could change depending on the variety, the time of year and the intensity of stress water.

At the later stages of its vegetative phase (50 DAT), the water deficit for a period of 15 days causes severe stress, with values of water potential lower than -1.60 MPa, which decrease the yield. The categorization of water deficit stress based on leaf water potential in rice, makes it possible to specify that when plants reach -1.20 MPa, regardless of the developmental stage at which they are, the water sheet must be restored to achieve the recovery of the plants and to reach the highest yields.

The categorization of stress due to water deficit is a tool of high scientific value for the specific case of rice, since this plant has the adaptive capacity to tolerate the presence of a sheet of water throughout its biological cycle and is highly susceptible to water deficit caused by lack of irrigation.

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