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# RECOVERY OF THE RICE PLANT TO THE SUSPENSION OF THE WATER LAMINA. PART II

## Recuperación de la planta de arroz a la suspensión de la lámina de agua. Parte II

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**ABSTRACT**. Achieving an efficient use of irrigation water in rice cultivation, not only responds to the management of irrigation, also depends on the response of the plant to physiological and biochemical level of it, to adapt and recover from the water deficit and to complete its biological cycle. The experiment was carried out at the Zaidín Experimental Station, Granada, Spain, under semi-controlled conditions in plastic pots, with 'INCA LP-5' rice plants, which were cultivated under anaerobic conditions and exposed to water deficit, the suspension of the water lamina at three stages of its development, at 30, 40 and 50 days after transplantation (DAT) for a period of 15 days and evaluated after the recovery period at 122 DAT. In general, plants recovered from the water stress to which they were exposed during their vegetative phase, which was evidenced through increased water potential and stomatal conductance, also increased the content of hydrogen peroxide and oxidative damage to lipids. In addition, a direct relationship was found between these variables (hydric potential - stomatal conductance and hydrogen peroxide - oxidative damage to lipids). These variables indicated that plants exposed to water stress at 30 DAT, showed a higher recovery state than those exposed to 40 and 50 DAT, response may influence crop yield.

*Key word*: water stress, *Oryza sativa*, hydrogen peroxide, lipid peroxidation,

**RESUMEN**. Lograr un uso eficiente de agua de riego en el cultivo del arroz, no responde únicamente al manejo del riego, también depende de la respuesta de la planta a nivel fisiológico y bioquímico de la misma, para adaptarse y recuperarse del déficit hídrico y llegar a completar su ciclo biológico. La investigación se realizó en la Estación Experimental del Zaidín, Granada, España, en condiciones semi-controladas en macetas plásticas, con plantas de arroz `INCA LP-5´, que se cultivaron en condiciones de anaerobiosis y fueron expuestas a déficit hídrico, mediante la suspensión de la lámina de agua en tres momentos de su desarrollo, a los 30, 40 y 50 días después del trasplante (DDT) por un período de 15 días y se evaluaron después del período de recuperación a los 122 DDT. En general, las plantas se recuperaron del estrés hídrico al que estuvieron expuestas durante su fase vegetativa, lo que se evidenció a través del aumento del potencial hídrico y la conductancia estomática, también se incrementó el contenido de peróxido de hidrógeno y el daño oxidativo a los lípidos. Además, se encontró una relación directa entre estas variables (potencial hídrico - conductancia estomática y peróxido de hidrógeno - daño oxidativo a lípidos). Estas variables indicaron que las plantas expuestas a estrés hídrico a los 30 DDT, mostraron un estado de recuperación mayor que las expuestas a los 40 y 50 DDT, respuesta que puede influir en el rendimiento agrícola del cultivo.

*Palabras clave*: estrés hídrico, *Oryza sativa*, peróxido de hidrógeno, peroxidación lipídica,

## NTRODUCCIÓN

The worldwide variability in rice crop yield is influenced by climate changes during the last three decades, and it was estimated that approximately 53 % of the regions where rice is harvested, experience the influence of this climatic phenomenon, with 32 % of the rice yield variability per year (1). The drought limits the production of rice, because it affects morphological level (reduction of germination, plant biomass, number of stems, various traits of roots and leaves) and physiological (reduced photosynthesis, perspiration, stomatal conductance, chlorophyll content, activity of photosystem II and the relative water content).

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Also at biochemical level modifies the accumulation of osmoprotectives like, the proline, sugars, polyamines and antioxidant compounds, as well as the hormonal production (auxins, cytokinins, gibberellins, ethylene and abscisic acid).

Rice (*Oryza sativa* L.) to be cultivated requires a large amount of water throughout its life cycle compared to other crops, even when it is not an aquatic plant. Therefore, drought stress causes a serious threat to rice production (2). The drought is recognized as an environmental disaster that harms the production of rice. Improving tolerance to drought in rice is a difficult task due to its complex and unpredictable nature (2).

Water deficit modifies the physiology of the rice plant in countless ways, such as net photosynthesis (3), transpiration rate (4), stomatal conductance (5), and efficiency of water use (6), content relative water (7) and the stability index of the membrane (8); and at a biochemical level the contents of reactive oxygen species (3). All these parameters are indicators of water stress in rice (6,8) To facilitate the development of tolerant varieties that can survive and give higher yields in drought conditions, it is necessary a deep knowledge of the diverse morphological, physiological and biochemical characters that govern the yield of rice in conditions of water stress. The objective of this work is to evaluate the recovery of rice plants at a physiological and biochemical level cultivated in anaerobic conditions and exposed to suspension of the water lamina for a period of 15 days in the vegetative phase.

## MATERIALS AND METHODS

The research was carried out in the Experimental Station of Zaidín, Granada, Spain (EEZ) in 2010 under greenhouse conditions, with rice plants cv. INCA LP-5.

Initially a rice seedbed was established in plastic trays of 0,40x0,80x0,08 m with sterile sand. To achieve the germination of the rice, the trays were irrigated until a lamina of water of 5 cm was obtained above the surface of the sand for a period of 24 hours. Moment from which the tray was drained, maintaining the sand at maximum water retention capacity, until two leaves sprouted per plant. Subsequently, the 3 cm water lamina was restored up to 30 days after the emergency (DAE).

At 30 DAE a plant was transplanted in each pot of 1 kg capacity (0.18 m high and 0.13 m in diameter), which contained 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 at 120 °C for 20 min, in a Selecta autoclave, model PRESOCLAVE-II 75 L, and the soil at 95-100 ºC, but for 60 minutes daily for three consecutive days. The soil that was used was classified as Fluvisol Haplic Calcaric (9), the one that presented a pH of 8.1 (measured by potentiometry), 1.81 % of organic matter (Walkley and Black method), assimilable phosphorus 6.2 mg $kg^{-1}$ (P-Olsen) and interchangeable potassium 0.34 cmol $kg^{-1}$ (extraction with 1 mol $NH_4OAc$ $L^{-1}$ at pH 7).

The pots were placed in the greenhouse where the seedbed was established, with temperatures of 26 and 22 ºC (day/night, respectively); relative humidity between 50-70; 16-hour light photoperiod 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 a completely randomized experimental design, with bifactorial arrangement and five repetitions, for which 15 pots were used per treatment, which allowed the evaluations to be carried out after each period without a lamina of water.

The water supply consisted in maintaining a lamina of water at 5 cm above the surface of the substrate in all treatments (Without E), until the moment when the lamina of water was suspended, at 30, 40 and 50 days after the transplant (With E), for a period of 15 days, at which time the lamina of water that remained until 15 days before harvesting was replaced; the group of pots to which the water lamina was not suspended remained as control treatments.

The total application of nutrients, corresponding to 0.123 g of N; 0.050 g of $P_2O_5$ and 0.059 g of $K_2O$ per pot, was carried out at 20, 35 and 60 days after transplantation (DAT), applying at each time 30, 40 and 30 %, respectively, using as carriers, urea (46 % N), triple superphosphate (46 % $P_2O_5$) and potassium chloride (60 % $K_2O$), respectively.

### Sampling and evaluations

Five plants were taken per treatment at 122 DAT (25 days before the grain harvest), to evaluate the height of the plants (ALT), the fresh air mass (MFA) and the roots (MFR), the agricultural yield and its components, the potential foliar hydric (Ψh), stomatal conductance (CE), foliar contents of hydrogen peroxide ($H_2O_2$) and oxidative damage to lipids (DOL).

The ALT, MFA, MFR, Ψh, CE, $H_2O_2$ and the DOL, were evaluated following the same procedures and protocol that are described in the first part of this article (10).

The means of the treatments were compared from the confidence intervals for α = 0.05. Regression analysis was also carried out between the hydric potential and the stomatal conductance, and between the content of hydrogen peroxide ($H_2O_2$) and the oxidative damage to lipids and it.

## RESULTS AND DISCUSSION

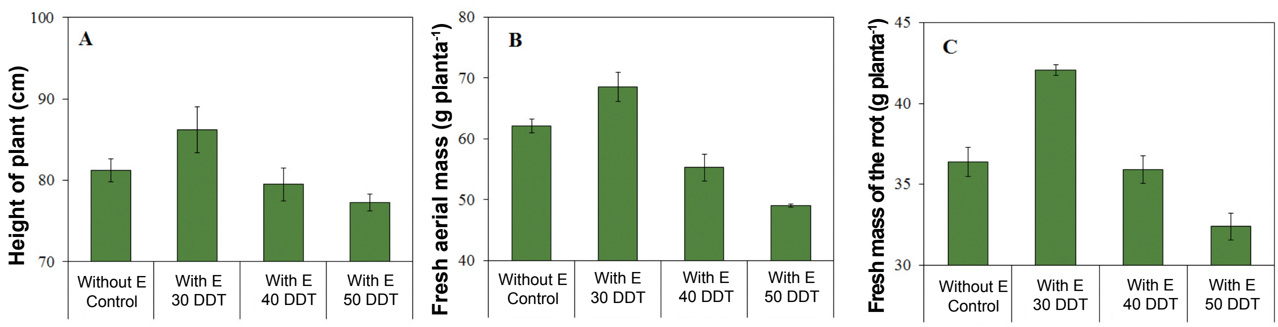
The water stress imposed at 30 DAT caused higher ALT, MFA and MFR after the recovery period (122 DAT), on the contrary, this condition at 40 DAT showed no differences with the control, always the growth and development of rice limited with the imposition of the water deficit to the 50 DAT (Figure 1A, B and C).

The condition of water stress at 30 DAT, limited the growth and development of the plant (11); however, at 122 DAT the same increased, which could be related to the time that elapsed since the plants concluded this stress period and the time of evolution at 122 DAT (recovery period) which was lower in those plants that were exposed later to this condition and, on the other hand, with the phenological state of the plant at the time of imposing the reduction of water supply. This response indicated the potential of the rice plant to recover after a period of water stress, since the growth of the plants increased at a higher rate, a behavior that corresponded to what was reported for this crop, but cultivated by direct seeding (12,13). The recovery of the rice plant is possible as long as the period of water stress does not categorize as severe stress (14).

At 122 DAT (Figure 2A), the plants that were exposed to hydric stress the $\Psi_h$ and the EC presented higher values, with respect to the evaluations made at 45, 55 and 65 DAT, that is, after each period of stress ended water (10).

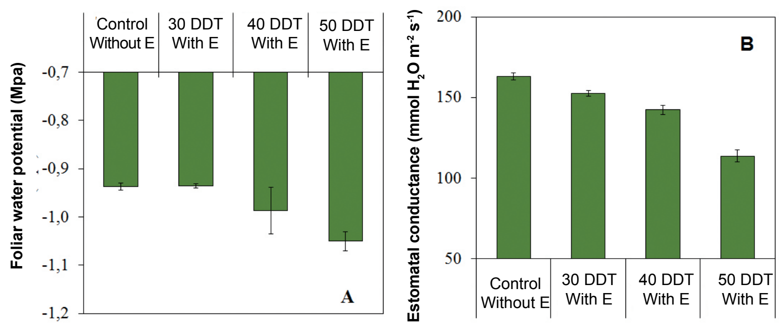
The $\Psi_h$ and EC were always lower in the treatment plants With E at 40 and 50 DAT.

It is important to note that after the recovery period, the recovery capacity of the plants, exposed to water stress, was observed through Ψh. In this regard, it was reported that rice has the capacity to recover, even though the water stress imposed can be considered moderate due to its duration and intensity (12).




Bars on the columns indicate Confidence intervals (α = 0.05)

**Figure 1. Height (A), fresh aerial mass (B) and fresh mass of the root (C), of rice plants cultivated with lamina of water (Without E) and exposed to suspension of the same (With E) for a period of 15 days, at 30, 40 and 50 DAT, respectively**




Bars on the columns indicate Confidence intervals (α = 0.05)

**Figure 2. Foliar water potential (A) and stomatal conductance (B), in rice plants cultivated with lamina water (Without E) and exposed to suspension thereof (With E) for a period of 15 days, at 30, 40 and 50 DAT, respectively**

On the other hand, the EC also indicated a recovery of the plants, even though they have not exceeded the EC values reached by the control treatment, this result suggests that the damage caused by the water stress in the leaves should cause variations in the number and stoma functionality. In addition, it became more evident in those treatments exposed to water stress at 40 and 50 DAT, response that had an effect on ALT and MFA accumulation, since this did not exceed the treatment of 30 DAT and the control.

The regression analysis between the $\Psi_h$ and CE showed a coefficient of determination of $R_2$=0.8368 and that of correlation is 0.914, indicating a direct linear relationship between the variables, statistically significant for a confidence level of 95 %, it was appreciated that as the water potential increased, stomatal conductance was also increased (Figure 3).

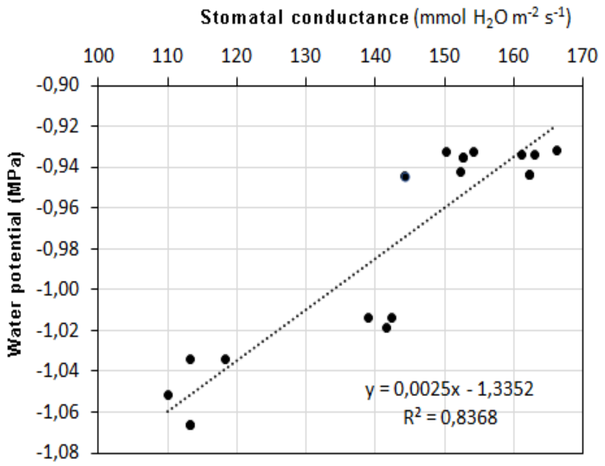


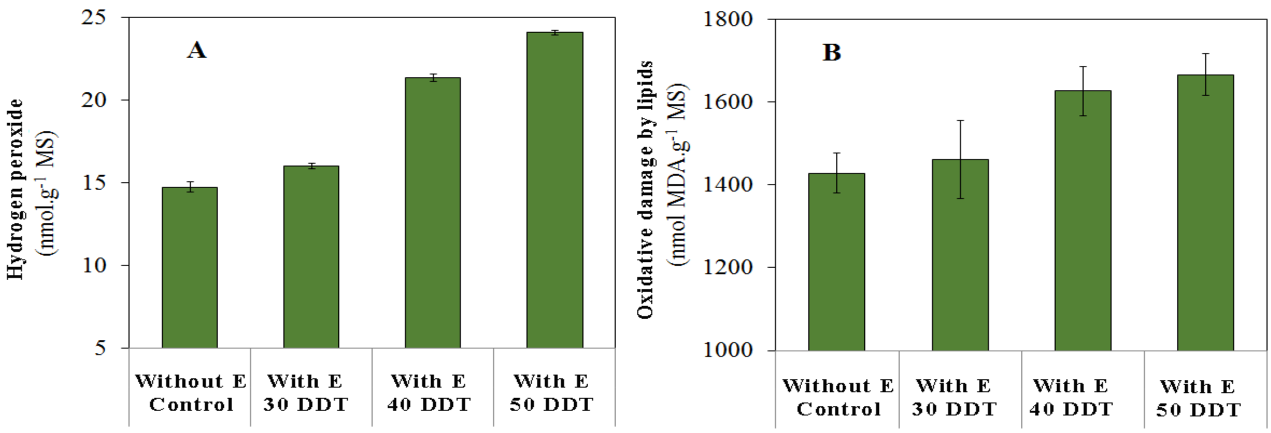

**Figure 3. Regression between the water potential and the stomatal conductance of plants cultivated with lamina water (Without E) and exposed to suspension thereof (With E) for a period of 15 days, at 30, 40 and 50 DAT, respectively**

The relationship found between the $\Psi_h$ and CE corroborates the considerations made previously when analyzing Figure 2 (A and B), even though the moment in which water stress was applied during the vegetative phase of the crop is decisive. The recovery of the plants through these two variables ($\Psi_h$ and CE), may be due to the rehydration of all tissues, as well as to the progressive activation of the processes of photosynthesis and respiration, in addition to improving the active transport of water and nutrients in the plant, which decreased as a defense mechanism against water stress (15,16). The response found in terms of ALT, MFA, MFR, $\Psi_h$ and CE showed that the rice plant activates evasion mechanisms in the face of a stress condition (17,18) and they are able to recover, provided that the intensity of the stress water does not totally cause cell death.

The exposure to water stress during the vegetative phase at 30, 40 and 50 DAT, increased the content of $H_2O_2$ and DOL, with respect to the controls, contents that at 122 DAT continued to be higher in these plants, with the exception of the DOL of those that were exposed to 30 DAT, which did not show differences with the control (Figure 4A and B).

From the biochemical point of view, the plants that were exposed to water stress during the vegetative period showed a differential behavior, which is very much associated with the moment in which the evaluation was carried out, that is after a recovery period (at 122 DAT).

On the other hand, it is important that the moment of evaluation (122 DAT, 25 days before the harvest), the plants were in a process characterized by a greater demand of water for the translocation of metabolites and filling of the grains (14), as well as the increase in senescence processes, the latter aspect that decreases photosynthesis (19) and causes biochemical changes in the plant in order to complete its biological cycle during the maturation phase.




Bars on the columns indicate Confidence intervals (α = 0.05)

**Figure 4. Contents of foliar hydrogen peroxide (A) and oxidative damage by lipids (B), rice plants cultivated with water lamina (Without E) and exposed to suspension thereof (With E) for a period of 15 days , at 30, 40 and 50 DAT, respectively**

The regression analysis between $H_2O_2$ and DOL showed a determination coefficient of $R_2$ = 0.8123 and that of correlation is 0.901, indicating a direct linear relationship between the variables, statistically significant for a confidence level of 95 %, it was observed that as the content of foliar hydrogen peroxide increased, it also increased oxidative damage to lipids (Figure 5).

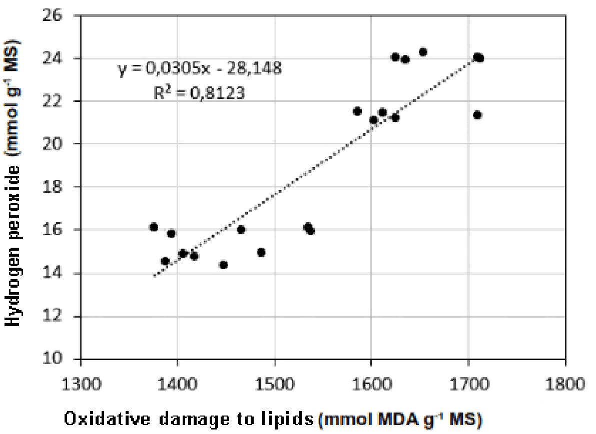



**Figure 5. Regression between the content of foliar hydrogen peroxide and the oxidative damage to lipids of rice plants cultivated with water lamina (Without E) and exposed to suspension thereof (With E) for a period of 15 days, a 30, 40 and 50 DAT, respectively**

The relationship found between $H_2O_2$ and DOL corroborates what has been reported by other authors regarding the increase in lipid peroxidation, that is, damage to lipids in the face of an abiotic stress condition (20). ), although this affectation can be caused by other reactive oxygen species (ROS), among which is the hydroxyl radical ($OH^-$), the superoxide ($O_2^-$) and the singlet oxygen ($^1O_2$). In the case of ROS, they are considered molecules indicating abiotic stress, and in the case of $H_2O_2$, it performs several functions, as an indicator and as a signal molecule (20). $H_2O_2$ is not only the most stable ROS with the ability to easily diffuse from one cell compartment to another, it can also be easily assimilated by an efficient cellular antioxidant system, because it occurs at high rates under drought conditions (21). In this work in the maturation phase of the rice crop, it is possible that the $H_2O_2$ content found (20.07 nmol $g^{-1}$ MS), is not exercising the function of stress signaling, if we take into account the values that are they found at the end of each period of water stress (average 17.88 nmol $g^{-1}$ MS), which indicated a water deficit in the plant (10). It is possible that at this moment they are expressing concentrations related to the process of senescence and aging of the leaves. In general, there is no clear picture of when we are in the presence of a signaling process or phytotoxicity from the $H_2O_2$ contents and the oxidative damage caused. It is possible that the values found for $H_2O_2$ and DOL are associated with the plant's own biology in the maturation phase.

## CONCLUSIONS

The plants recovered from the water stress to which they were exposed during their vegetative phase, which was evidenced through the increase of water potential and stomatal conductance, the content of hydrogen peroxide and oxidative damage to lipids was also increased. In addition, a direct relationship was found between the potential water variables - stomatal conductance and hydrogen peroxide - oxidative damage to lipids. These variables indicated that plants exposed to water stress at 30 DAT, showed a recovery state higher than those exposed to 40 and 50 DAT, response that can influence agricultural performance

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