ISSN impreso: 0258-5936
ISSN digital: 1819-4087
Ministerio de Educación Superior. Cuba
Instituto Nacional de Ciencias Agrícolas
http://ediciones.inca.edu.cu

RESPONSE OF RICE (*ORYZA SATIVA* L.) PLANT TO SUSPENSION OF THE WATER LAMINA IN THREE MOMENTS OF ITS DEVELOPMENT. PART I

Respuesta de la planta de arroz (*Oryza sativa* L.) A la suspensión de la lámina de agua en tres momentos de su desarrollo. Parte I

Michel Ruiz Sánchez[1]✉, Yaumara Muños Hernández[2], José M. Dell'Ámico Rodríguez[1], Juan A. Cabrera Rodríguez[1], Ricardo Aroca[3] and Juan M. Ruiz Lozano[3]

ABSTRACT. The efficient use of irrigation water in rice cultivation is a priority, due to the volume of water consumed this grain; for this reason the application of alternatives that reduce the volumes of water to use is necessary, without it affecting the agricultural performance. The research was conducted at the Experimental Station of Zaidín, Granada, Spain, in semi-controlled plastic flowerpots conditions with rice plants `INCA LP-5', which were cultured under anaerobic conditions and were exposed to water deficit by the suspension of the water lamina in three stages, at 30, 40 and 50 DAT for a period of 15 days. It was evident that the suspension of the lamina of water caused water stress on the plant, which manifested itself through decreased air plant growth, leaf water potential and stomatal conductance. On the contrary, the plant root mass, the content of proline, hydrogen peroxide, oxidative damage and antioxidant glutathione content increased, while the reduced ascorbate decreased. In general, the water deficit worsened in the plant when applied to the 50 DAT, regarding the suspension of the water level at 30 DAT.

Key words: antioxidant, stomatal conductance, floow, water, potential hydric

RESUMEN. El uso eficiente del agua de riego en el cultivo del arroz es prioritario, debido a los volúmenes de agua que consume este cereal; por tal motivo se hace necesario la aplicación de alternativas que disminuyan los volúmenes de agua a utilizar, sin que se afecte el rendimiento agrícola. 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, a los 30, 40 y 50 DDT por un periodo de 15 días. Se evidenció que la suspensión de la lámina de agua provocó un estrés hídrico en la planta, que se manifestó a través de la disminución del desarrollo aéreo de la planta, el potencial hídrico foliar y la conductancia estomática. Por el contrario, se incrementó la masa radical de la planta, el contenido de prolina, de peróxido de hidrógeno, el daño oxidativo y el contenido del antioxidante glutation, a la vez que disminuyó el ascorbato reducido. En sentido general, el déficit hídrico en la planta se agudizó cuando se aplicó a los 50 DDT, respecto a la suspensión de la lámina de agua a los 30 DDT.

Palabras clave: antioxidante, conductancia estomática, inundado, irrigación, potencial hídrico

INTRODUCTION

In Cuba, rice is grown in three different conditions depending on the water applied. The first condition is with irrigation or flooding, which needs sufficient availability of water to guarantee the cultivation, in all or in great part of its cycle, with a lamina of water of approximately 10 cm. The second is known as rainfed rice, depending on rainfall throughout its biological cycle, which requires as much as 200 mm of rain per month and it is important both the amount and frequency of rainfall. Under these conditions, this cereal is severely affected by the lack of water throughout its cycle,

[1] Instituto Nacional de Ciencias Agrícolas (INCA), gaveta postal 1, San José de las Lajas, Mayabeque, Cuba, CP 32 700
[2] Universidad Hermanos Saiz Montes de Oca
[3] Estación Experimental del Zaidín, Granada, España
✉ mich@inca.edu.cu

when there is no abundant precipitation or stable sources of supply. Finally, rice cultivation in favored rainfed conditions is also dependent on rainfall, but occasionally receives irrigation water supply (1). Although rice is grown under the three conditions mentioned above, the permanent crop represents the largest area (2) and yields still do not exceed 3,5 t ha^{-1} as a national average (1).

Since the 1990s, research on water management was carried out through the application of a water deficit (water stress) in the vegetative phase in rice cultivation by direct sowing, which demonstrated the increase in agricultural yield[A]; results that were included in the Technical Standards for Crops 2005. However, these investigations did not deepen the physiological and biochemical response at the plant level and did not include the rice production by transplant technology. The rice cultivated by this technology consumes less water than the rice that is cultivated by direct sowing (3); however, it has not been reported for this technology the moment that a water deficit can be applied, as an alternative to save irrigation water and increase agricultural yield.

In Cuba, several studies have been carried out to study the effect of induced water deficit in the early stages of rice growth, to identify physiological variables that facilitate the selection of tolerant varieties and to know the contribution of stem carbohydrates in the filling of grains by direct sowing (4).

A possible way to increase the rice yield, with a greater efficiency in the use of water, could be the application of water stress during the vegetative phase. The objective of this first part is to evaluate the physiological and biochemical response of rice cultivated under anaerobic conditions and exposed to suspension of the water table 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 cv. INCA LP-5.

Initially a seedbed of rice was established in plastic trays of 0,40 x 0,80 x 0,08 m with sterile sand. To achieve the rice germination, the trays were watered until a lamina of water 5 cm above the surface of the sand was obtained for a period of 24 hours, at which time the tray was drained, keeping the sand at maximum water retention capacity, until two leaves per plant emerged. Subsequently, the 3 cm water lamina was reestablished until 30 days after the emergency (DAE).

One plant was transplanted to 30 DAE in each pot of 1 kg of capacity (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). The sand was sterilized at 120 °C for 20 min in a Selecta autoclave, model PRESOCLAVE-II 75 L, and the soil at 95-100 °C for 60 min daily for three consecutive days. The soil that was used was classified as Fluvisol Haplic Limestone (5), which presented a pH of 8,1 (measured by potentiometry), 1,81 % of organic matter (6), assimilable phosphorus 6,2 mg kg^{-1} (P-Olsen) and exchangeable potassium 0,34 cmol kg^{-1} (extraction with NH_4OAc 1 mol L^{-1} at pH 7).

The pots were placed in the greenhouse where the seedlings were established, with temperatures of 26 and 22 ºC (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 LIICOR (Lincoln, NE, USA, model LI-188B); Following a completely randomized experimental design, with bifactorial arrangement and five replicates. Fifteen pots per treatment were used, which allowed to carry out the evaluations after each period without water lamina.

The water supply consisted in maintaining a water lamina of 5 cm on the surface of the substrate in all the treatments. Fifty percent of the pots were suspended at 30, 40 and 50 days before transplantation (DBT), for a period of 15 days. At which time the water lamina was replenished until 15 days before harvesting. The group of pots that did not suspend the water sheet remained as control treatments.

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, 35 and 60 DAT at 30, 40 and 30 %, respectively, using Urea (46 % N), Triple Superphosphate (46 % of P_2O_5) and Potassium Chloride (60 % of K_2O), respectively.

[A] Polón, R. "Impacto nacional en el incremento del rendimiento agrícola, economizar agua de riego y energía en el cultivo del arroz (*Oryza sativa* L.) como consecuencia del estrés hídrico" [en línea]. En: *XVI Fórum de Ciencia y Técnica*, 2007, Código: 0109604 02, [Consultado: 1 de diciembre de 2007], Disponible en: <http://www.forumcyt.cu/UserFiles/forum/Textos/0109604.pdf>.

Sampling and evaluations carried out

Five plants per treatment were taken 15 days after the suspension of the water lamina (45, 55 and 65 DAT), to evaluate the height of the plants (AP), fresh aerial mass (AFM) and roots (MFR), agricultural yield and its components, leaf water potential (Ψh), stomatal conductance (EC), proline foliar contents (PRO), hydrogen peroxide (H_2O_2), lipid oxidative damage (DOL), reduced ascorbate (ASC) content, reduced glutathione (GSH), and mycorrhizal root colonization.

The AP was measured from the surface of the substrate to the upper end of the longest leaf projected in the same direction of the stem. The MFA and the MFR were determined separately, through a cut that was made at the base of the stem, the masses of both were measured in a Technical Scale (Denver Instrument PK-601, with a margin of error of 0,01 g) and expressed in g $plant^{-1}$.

The Ψh was determined by the Psycrometric method (7), two hours after the start of daytime photoperiod (between 9:00 and 10:00), for which a system integrated by the microvoltmeter HR-33T connected to a camera Psychotherapeutic C52 (Wescor Inc., Logan, UT, USA). Ten discs (0,005 m Diameter) by treatment of leaf central part of the plant and placed in the chamber for 15 min to stabilize the temperature and the water steam of the disk before performing the reading of Ψh, which was expressed in MPa.

The EC was measured using an AP4 Portion (Delta-T Devices Ltd, Cambridge, UK), at the same times where Ψ_h was determined and expressed in mmol H_2O m^{-2} s^{-1}. Both variables were measured in the well-developed leaves of the upper third of the plant.

For the biochemical analyzes of PRO, H_2O_2, DOL and GSH, three samples each of 0.5 g per plant and 0.2 g per plant for the ASC were taken, which were frozen at the time of sampling with liquid nitrogen and stored at -80 °C.

After macerating the samples in liquid nitrogen and adding the extraction solution specific for each variable (Table), centrifuged or filtered as appropriate and the supernatant (extract) was extracted. The precipitate from each extraction was collected in sterile Eppendorf, previously weighed to determine the dry mass (MS). In all cases the absorbance was measured by molecular absorption spectrophotometry (Hitachi, model U-1900, Japan).

The PRO was determined at 530 nm of absorbance (8), using ninhydrin reagent. A standard curve was made from a proline solution (1 mM) with the following concentrations: 0, 25, 50, 200, 200 and 300 µM) of proline.

The content of hydrogen peroxide was determined in the leaves (9), at 508 nm absorbance. As a blank, 5 % TCA was used instead of extract and the standard curve was prepared with H_2O_2 dissolved in extraction solution (Table). The H_2O_2 content was expressed in nmol g^{-1} MS.

Oxidative lipid damage was determined by reading the absorbance at 532 and 600 nm (10), this parameter was estimated from the content of reactive substances of thiobarbituric acid and expressed as MDA equivalents (11). As a blank extraction solution was used instead of extract (Table) and for the standard curve was prepared with malondialdehyde (MDA) dissolved in extraction solution, in the range of 0,1-10 nmol. The DOL was expressed in nmol MDA g^{-1} MS. The ASC content was quantified at 524 nm (12). Extraction solution was used as the blank instead of extract (Table). The reduced ascorbate content was expressed in nmol g-1 MS. The GSH content was determined at 412 nm for 5 min with a 1 min interval (13). As a white extraction solution was used instead of extract (Table) and for the standard curve oxidized glutathione (GSSG) dissolved in extraction solution was used. The reduced glutathione content was expressed in nmol g^{-1} MS.

The means of the treatments were compared from the confidence intervals for α = 0,05.

Table. Conditions and procedures of plant extraction for the determination of the biochemical variables analyzed

Variables	Extraction Solutions	Volume of extraction	Centrifuged or filtered
Hydrogen peroxide	Trichloroacetic acid (TCA) 5% (m / v) + activated carbon 2% (m / v) + polyvinyl polypyrrolidone (PVPP) 2% (m / v)	5 mL	18 000 g for 10 min at 4 ºC
Oxidative lipid damage	Trichloroacetic acid (TCA) 15% (w / v)	4 mL	1500 g for 20 min at 4 ° C
Reduced Ascorbate	Metaphosphoric Acid 2% (m / v) + NaCl 20% (m / v)	5 ml	Filtered with filter paper
Reduced glutathione and proline	Sulphosalicylic acid 5% (w / v)	5 mL	1000 g for 10 min at 4 ºC

RESULTS AND DISCUSSION

The suspension of the water lamina (Con E) conditioned the cracking of the substrate in the pot, besides the curl and erection of the leaves, evidencing a water deficit in the plant, in the three moments that the water lamina was suspended (Figure 1).

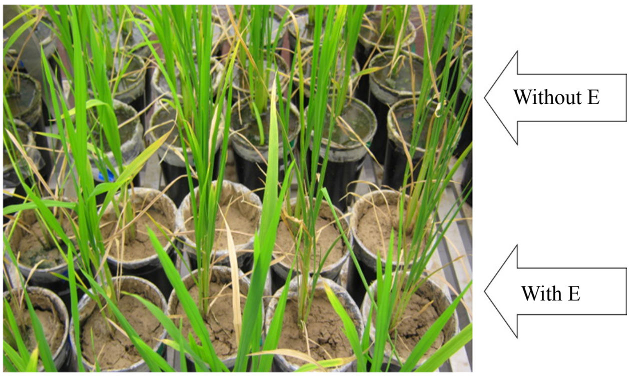


Figure 1. Effects of the suspension of the water lamina for a period of 15 days at the 30 DAT, under potting conditions

Behavior of other physiological indicators in plants

Figure 2 shows the effect on height (AP), accumulation of fresh mass of aerial part (MFA) and roots (MFR) of rice plants that remained with a water lamina from the transplant time (without E) and that they were exposed to suspension of the water lamina (Con E) for a period of 15 days, at 30, 40 and 50 DAT.

Plants exposed to water lamina suspension showed lower AP (Figure 2A) and MFA (Figure 2B). In the case of the MFR, the highest values were presented in the plants exposed to suspension of the water lamina in the three moments that this condition was imposed (Figure 2C).

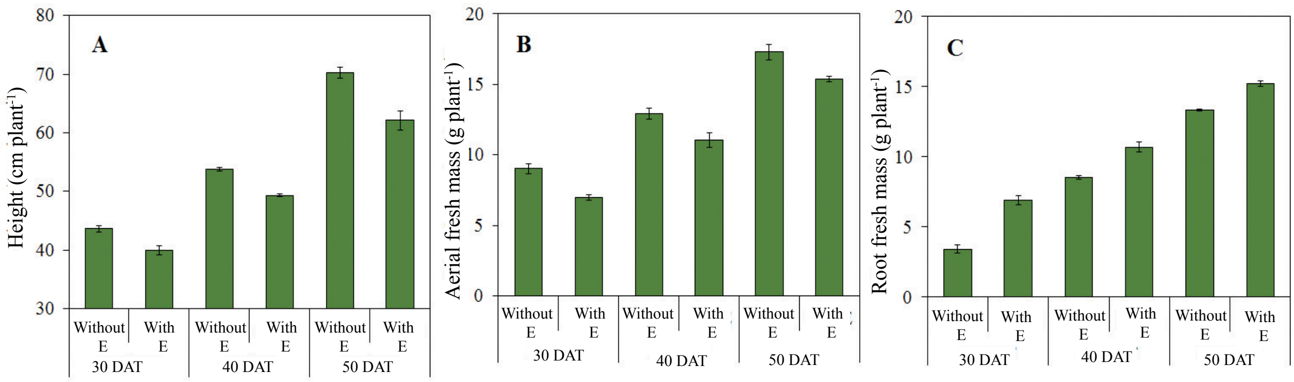
The potential leaf water variable (Ψ_h) and stomatal conductance (EC) are shown in Figure 3. The period without a water slide caused a water stress in the plant, which increased as the stress was imposed later, reflected this in a decrease of Ψ_h (Figure 3A).

The plants that were exposed to suspension of the water lamina in the three moments, evidenced water deficit, reflected this in a decrease of the EC (Figure 3B). The EC was always higher in the control plants (Without E), with the highest values being found in those evaluated at 45 DAT. Under these conditions, no differences were observed at 45 and 55 DAT.

The reduction of Ψ_h and EC indicated a water deficit in plants caused by the reduction of water supply, variables that have been used as indicators of stress due to water deficit in other crops, such as tomato (14), *Sorghum bicolor* L. Moench (15), maize (16) and rice (3, 17).

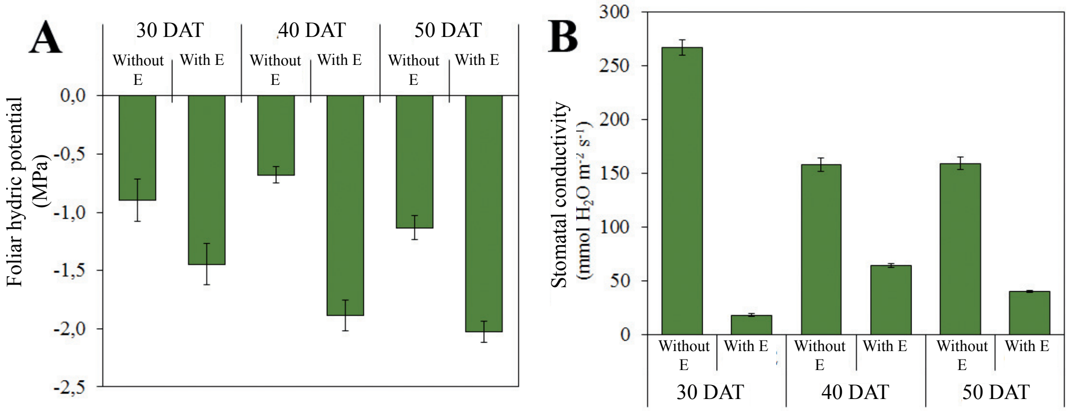
To the extent that water stress was imposed later, it was limited to the growth and development of the rice plant, which could be attributed to a decrease in the entry of CO_2 into the cells and a low efficiency in the use of the light; at the same time, could cause inactivation of the photosynthetic system and therefore, the plants did not produce sufficient assimilates that contributed to the cell division of the meristematic tissue for plant growth (18).

Similar results were reported when growing rice in non-flooded and flooded conditions (19). These same authors emphasized that rice is sensitive to soil water potentials below -30 KPa and besides that, water stress stimulates radical growth.



Bars on the columns indicate confidence intervals (α = 0,05)

Figure 2. Height (A), fresh aerial mass (B) and fresh root mass (C), in plants cultivated with water lamina (Without E) and exposed to suspension (with E) at 30, 40 and 50 DAT respectively



Bars on the columns indicate confidence intervals (α = 0,05)

Figure 3. Foliar hydric potential (A) and stomatal conductance (B), in plants cultivated with water lamina (Without E) and exposed to suspension (E) at 30, 40 and 50 DAT, respectively

Although this level of potential does not reduce transpiration in other rainfed crops, in rice it does occur (19), because it presents a high sensitivity in the stomata, which is related to the mechanisms of signaling of the root through the ABA to prevent water losses (20, 21).

All of the above indicated that the response found for Ψ_h and CE, is also related to the water absorption capacity of the plants and presumably to the hydraulic conductivity of the root; which apparently decreased the resistance to the passage of water, in spite of a greater growth of the roots, with respect to the control treatment (without E).

The ranges of Ψ_h found with the imposition of water stress for a period of 15 days, indicated that this was severe for those plants that were exposed to it at 40 and 50 DAT and from moderate to 30 DAT, according to the ranges proposed since the year 1973 (22). This proposal was based on an analysis of the published results on the effect of water stress on plants and defined three degrees of stress to categorize the water status of the plant, taking into account the values of Ψ_h and the relative water content (CRA):

- Light water deficit: when Ψ_h is greater than -5 bar (-0.5 MPa) and CRA is reduced by 8-10%.
- Moderate water deficit: Ψ_h is between -5 bar (-0.5 MPa) to -12 or -15 bar (-1,2 MPa or -1,5 MPa) and CRA decreases between 10 and 20 %.
- Severe water deficit: Ψ_h is less than -15 bar (-1.5 MPa) and CRA decreases by more than 20 %.

As a reference, the proposed categorization corroborates that, for treatments exposed to water deficit at 40 and 50 DAT, water stress was severe, probably due to biochemical changes during stress exposure (9), as will be analyzed below.

Behavior of biochemical indicators in plants

Figure 4 shows the content of proline (PRO), hydrogen peroxide (H_2O_2), oxidative lipid damage (DOL), reduced ascorbate content (ASC) and reduced glutathione (GLT) rice.

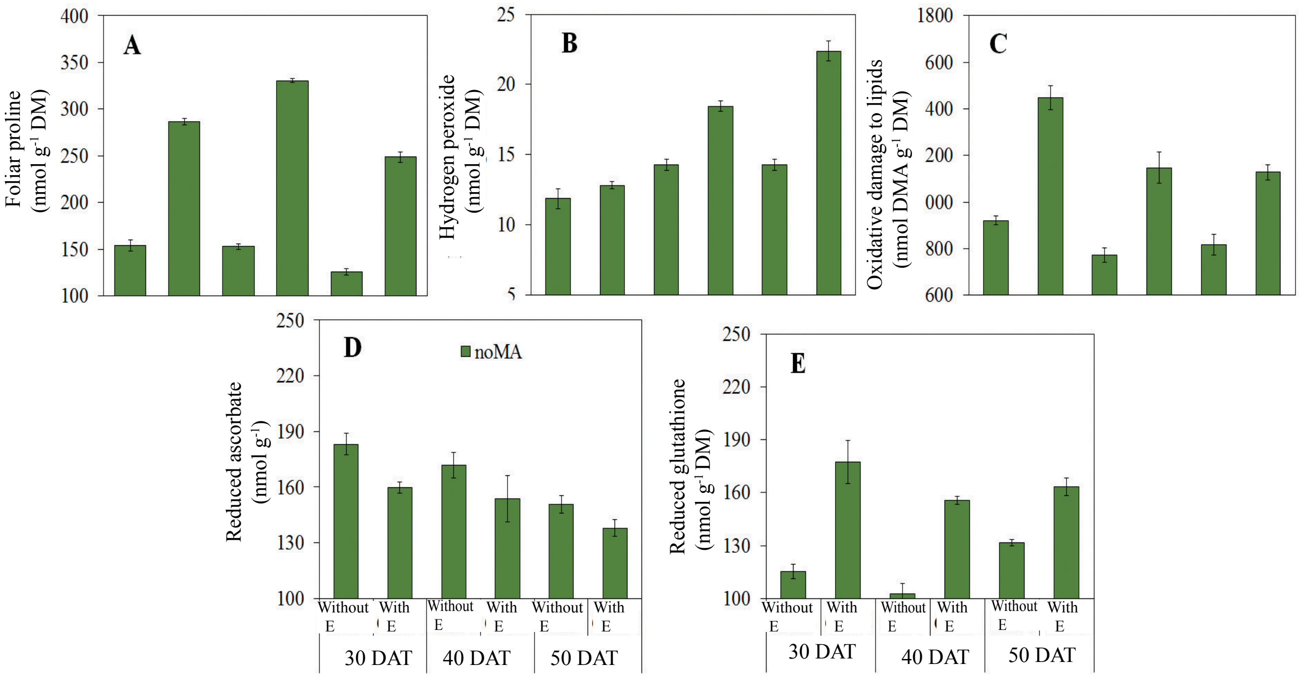
At each moment of evaluation, it was found that in the treatments without stress (Witness) the plants presented the smaller contents of PRO, this content decreased when they had a bigger growth and development (Figure 4A).

The contents of H_2O_2 at 30, 40 and 50 DAT were low in the control plants (without E) (Figure 4A), in relation to the treatments exposed to water stress (with E), in these the content of H_2O_2 in the as the stress was later applied (50 DAT).

DOL (Figure 4C) was lower in plants that were not exposed to stress due to water deficit (without E) and among them, it was higher when evaluating this indicator at 45 DAT. The higher values of DOL were found in plants exposed to water stress and within these treatments the imposition of this condition at 30 DAT presented higher values.

When analyzing the contents of the ASC non-enzymatic antioxidant molecule (Figure 4D), it was found increases in the control treatments at the three moments in which they were evaluated (45, 55 and 65 DAT) and among them the highest contents corresponded to the plants evaluated at 45 DAT.

On the other hand, in the treatments exposed to water stress the contents of ASC decreased as the stress was imposed later.



Bars on the columns indicate confidence intervals (α = 0,05)

Figure 4. Foliar Proline content (A), hydrogen peroxide content (B), oxidative lipid damage (C), reduced ascorbate content (D) and reduced glutathione content (E) in plants grown with water (without E) and exposed to suspension of the same (with E) at 30, 40 and 50 DAT, respectively

As for the GSH content (Figure 4E), the highest values were found in the plants of the stressed treatments, with values higher than the plants evaluated at 45 DAT (stress was imposed at 30 DAT).

Several authors report a high accumulation and increase of PRO synthesis under stress conditions, as an osmoregulatory and osmotic adjustment mechanism in plants exposed to abiotic stress (20,23), in addition to that the increase in PRO is directly related to the production of H_2O_2 (24).

The results found for PRO, at the end of the period of water stress (45 DAT) indicated that PRO may be expressed as a signifier of the condition to which the plants were exposed, even when expressed as an osmoprotective agent (19), or even doing both functions. These two mechanisms were reported by several authors (23, 24). PRO is an efficient indicator that is frequently used to assess the tolerance of plants to a condition of biotic or abiotic stress (4,8,16,17,23) and in the selection of cultivars tolerant to water stress or drought (24).

The increase of the GSH content corresponded to the decrease of the DOL and the content of H_2O_2 found in the rice plants. Therefore, it is possible to suggest its direct relation in the reduction of H_2O_2 to H_2O. Other antioxidant enzymes such as peroxidase (PRX), ascorbate peroxidase (APX), glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT), together with the ascorbate-glutathione cycle, are involved in the elimination of H_2O_2, in the defense mechanism of the plant before a water deficit (25).

Apparently given the levels of GSH found, its accumulation in plants is related to signals that regulate this reaction, because this contributes to the regeneration of ASC (26, 27).

Similar results were reported by other authors (17, 20), coinciding with a reduction of DOL and H_2O_2 content, when GSH content was increased, but in rice plants cultivated under aerobiosis conditions exposed to water deficit for a period of 15 days. Several authors have used DOL as an indicator in plants exposed to biotic or abiotic stress, as well as the content of hydrogen peroxide (25, 27-31).

The above allows interpreting that, under the conditions of anaerobiosis, the ASC is possible to perform functions such as the reduction of excess ROS over GSH or vice versa, within the ascorbate-glutathione mechanism. Another interpretation may be that, under these conditions, an increase in the enzymatic activity was observed, due to the oxidation or reduction of these two molecules (ASC and GSH), aspect not evaluated in this work and it is interesting to continue investigating this mechanism of Oxidation-reduction. In this regard, it was reported the need to accurately quantify the changes in the redox state within the cell during relatively mild oxidative stress (27), in order to quantify the changes recognized by the ROS that escape the antioxidant system and how many are modulated by the signal system (28).

The accumulation of ASC and GSH, in addition to counteracting the production of H_2O_2 in the plant, could be involved in the elimination of other ROS, such as free radicals, superoxide (O^{2-}), hydroxyl (OH-) And singlet oxygen ($^1O_2$).

CONCLUSIONS

- The suspension of the water lamina for a period of 15 days caused water stress in the plant, which was manifested through the decrease in plant growth and aerial development, leaf water potential and stomatal conductance, for on the contrary, the radical mass of the plant was increased.
- From the biochemical point of view, the content of proline, hydrogen peroxide, oxidative damage and antioxidant glutathione content was increased, while reduced ascorbate decreased.
- In a general sense, the water deficit in the plant was exacerbated when it was applied later (50 DAT), regarding the suspension of the water lamina at 30 DDT.

BIBLIOGRAPHY

1. Rivero, L. E. y Suárez, C. E. *Instructivo Técnico Cultivo de Arroz*. Ed. Asociación Cubana de Técnicos Agrícolas y Forestales, 2015, La Habana, Cuba, 73 p., ISBN 978-959-7210-86-3.
2. FAO. *Cuba* [en línea]. AQUASTAT - Sistema de Información sobre el Uso del Agua en la Agricultura de la FAO, 2016, [Consultado: 14 de febrero de 2017], Disponible en: <http://www.fao.org/nr/water/aquastat/countries_regions/cub/indexesp.stm>.
3. García, A. “Efecto del déficit hídrico sobre la distribución de fotoasimilados en plantas de arroz (*Orysa sativa* L.)”. *Interciencia*, vol. 35, no. 1, 2010, pp. 47–54, ISSN 0378-1844, [Consultado: 14 de febrero de 2017], Disponible en: <http://search.proquest.com/openview/af060b488c17e5fc7cf9b4c21b12a543/1?pq-origsite=gscholar&cbl=21011>.
4. Anup, D.; Patel, D. P.; Ramkrushna, G. I.; Munda, G. C.; Ngachan, S. V.; Choudhury, B. U.; Mohapatra, K. P.; Rajkhowa, R.; Kumar, D. J. y Panwar, A. S. *Improved Rice Production Technology – for resource conservation and climate resilience (Farmers’ Guide)*. Extension Bulletin, no. 78, Inst. ICAR Research Complex for NEH Region, 2012, Meghalaya, 33 p., ISBN 978-81-920769-5-9.
5. IUSS Working Group WRB. *World Reference Base for soil resources 2014: international soil classification system for naming soils and creating legends for soil maps*. (ser. World Soil Reports, no. ser. 106), Ed. Food and Agriculture Organization of the United Nations, 2014, Rome, 191 p., ISBN 978-92-5-108370-3.
6. Walkley, A. y Black, I. A. “An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method”. *Soil science*, vol. 37, no. 1, 1934, pp. 29–38, ISSN 0038-075X, 1538-9243, [Consultado: 22 de septiembre de 2016], Disponible en: <http://journals.lww.com/soilsci/citation/1934/01000/an_examination_of_the_degtjareff_method_for.3.aspx>.
7. Porcel, R. y Ruiz-Lozano, J. M. “Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress”. *Journal of Experimental Botany*, vol. 55, no. 403, 2004, pp. 1743-1750, ISSN 0022-0957, DOI 10.1093/jxb/erh188, [Consultado: 14 de febrero de 2017], Disponible en: <https://academic.oup.com/jxb/article/55/403/1743/531390/Arbuscular-mycorrhizal-influence-on-leaf-water>.
8. Bates, L. S.; Waldren, R. P. y Teare, I. D. “Rapid determination of free proline for water-stress studies”. *Plant and Soil*, vol. 39, no. 1, 1973, pp. 205-207, ISSN 0032-079X, 1573-5036, DOI 10.1007/BF00018060, [Consultado: 14 de febrero de 2017], Disponible en: <http://link.springer.com/article/10.1007/BF00018060>.
9. Aroca, R.; Irigoyen, J. J. y Sánchez-Díaz, M. “Drought enhances maize chilling tolerance. II. Photosynthetic traits and protective mechanisms against oxidative stress”. *Physiologia Plantarum*, vol. 117, no. 4, 2003, pp. 540-549, ISSN 1399-3054, DOI 10.1034/j.1399-3054.2003.00065.x, [Consultado: 14 de febrero de 2017], Disponible en: <http://onlinelibrary.wiley.com/doi/10.1034/j.1399-3054.2003.00065.x/full>.
10. Dhindsa, R. S.; Plumb-Dhindsa, P. y Thorpe, T. A. “Leaf Senescence: Correlated with Increased Levels of Membrane Permeability and Lipid Peroxidation, and Decreased Levels of Superoxide Dismutase and Catalase”. *Journal of Experimental Botany*, vol. 32, no. 1, 1981, pp. 93-101, ISSN 0022-0957, DOI 10.1093/jxb/32.1.93, [Consultado: 14 de febrero de 2017], Disponible en: <https://academic.oup.com/jxb/article-abstract/32/1/93/746156/Leaf-Senescence-Correlated-with-Increased-Levels>.
11. Halliwell, B. y Gutteridge, J. M. C. *Free radicals in biology and medicine* [en línea]. 2.ª ed., Ed. Clarendon Press, 1989, Oxford, 568 p., ISBN 978-0-19-855294-9, Google-Books-ID: Eok5AQAAIAAJ, [Consultado: 14 de febrero de 2017], Disponible en: <https://books.google.co.in/books?id=Eok5AQAAIAAJ>.

12. Leipner, J.; Fracheboud, Y. y Stamp, P. "Acclimation by suboptimal growth temperature diminishes photooxidative damage in maize leaves". *Plant, Cell & Environment*, vol. 20, no. 3, 1997, pp. 366-372, ISSN 1365-3040, DOI 10.1046/j.1365-3040.1997.d01-76.x, [Consultado: 14 de febrero de 2017], Disponible en: <http://onlinelibrary.wiley.com/doi/10.1046/j.1365-3040.1997.d01-76.x/full>.
13. Smith, I. K. "Stimulation of Glutathione Synthesis in Photorespiring Plants by Catalase Inhibitors". *Plant Physiology*, vol. 79, no. 4, 1985, pp. 1044-1047, ISSN 1532-2548, DOI 10.1104/pp.79.4.1044, [Consultado: 14 de febrero de 2017], Disponible en: <http://www.plantphysiol.org/content/79/4/1044>.
14. Fundora, L. R.; Mena, A.; Rodríguez, Y.; González, P. J.; Rodríguez, P. y González-Peña, D. "Estabilidad de la eficiencia de la cepa *Glomus mosseae* en la respuesta del tomate a condiciones de estrés hídrico fuera de su periodo óptimo". *Cultivos Tropicales*, vol. 29, no. 4, 2008, pp. 47-53, ISSN 0258-5936, [Consultado: 14 de febrero de 2017], Disponible en: <http://scielo.sld.cu/scielo.php?script=sci_abstract&pid=S0258-59362008000400007&lng=es&nrm=iso&tlng=es>.
15. Bárzana, G.; Aroca, R.; Paz, J. A.; Chaumont, F.; Martinez-Ballesta, M. C.; Carvajal, M. y Ruiz-Lozano, J. M. "Arbuscular mycorrhizal symbiosis increases relative apoplastic water flow in roots of the host plant under both well-watered and drought stress conditions". *Annals of Botany*, vol. 109, no. 5, 2012, pp. 1009-1017, ISSN 0305-7364, DOI 10.1093/aob/mcs007, [Consultado: 14 de febrero de 2017], Disponible en: <https://academic.oup.com/aob/article/109/5/1009/140064/Arbuscular-mycorrhizal-symbiosis-increases>.
16. Bárzana, G.; Aroca, R. y Ruiz-Lozano, J. M. "Localized and non-localized effects of arbuscular mycorrhizal symbiosis on accumulation of osmolytes and aquaporins and on antioxidant systems in maize plants subjected to total or partial root drying". *Plant, Cell & Environment*, vol. 38, no. 8, 2015, pp. 1613-1627, ISSN 1365-3040, DOI 10.1111/pce.12507, [Consultado: 14 de febrero de 2017], Disponible en: <http://onlinelibrary.wiley.com/doi/10.1111/pce.12507/full>.
17. Ruiz-Sánchez, M.; Geada, D.; Muñoz, H. Y.; Martínez, A.; Santana, Y.; Benítez, M.; Aroca, R. y Ruiz-Lozano, J. M. "La simbiosis micorrízica arbuscular en plantas de arroz (*Oryza sativa* L.) sometidas a estrés hídrico. Parte II Respuesta bioquímica". *Cultivos Tropicales*, vol. 36, no. 3, 2015, pp. 88-95, ISSN 0258-5936, [Consultado: 14 de febrero de 2017], Disponible en: <http://scielo.sld.cu/scielo.php?script=sci_abstract&pid=S0258-59362015000300014&lng=es&nrm=iso&tlng=es>.
18. Zubaer, M. A.; Chowdhury, A. K. M. M. B.; Islam, M. Z.; Ahmed, T. y Hasan, M. A. "Effects of water stress on growth and yield attributes of aman rice genotypes". *International Journal of Sustainable Crop Production*, vol. 2, no. 6, 2007, pp. 25-30, ISSN 1991-3036, [Consultado: 14 de febrero de 2017], Disponible en: <https://www.cabdirect.org/cabdirect/abstract/20083185007>.
19. Kato, Y. y Okami, M. "Root growth dynamics and stomatal behaviour of rice (*Oryza sativa* L.) grown under aerobic and flooded conditions". *Field Crops Research*, vol. 117, no. 1, 2010, pp. 9-17, ISSN 0378-4290, DOI 10.1016/j.fcr.2009.12.003, [Consultado: 14 de febrero de 2017], Disponible en: <http://www.sciencedirect.com/science/article/pii/S0378429009003335>.
20. Tseng, I.-C.; Hong, C.-Y.; Yu, S.-M. y Ho, T.-H. D. "Abscisic Acid- and Stress-Induced Highly Proline-Rich Glycoproteins Regulate Root Growth in Rice". *Plant Physiology*, vol. 163, no. 1, 2013, pp. 118-134, ISSN 1532-2548, DOI 10.1104/pp.113.217547, [Consultado: 14 de febrero de 2017], Disponible en: <http://www.plantphysiol.org/content/163/1/118>.
21. Augé, R. M.; Toler, H. D. y Saxton, A. M. "Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis". *Mycorrhiza*, vol. 25, no. 1, 2015, pp. 13-24, ISSN 0940-6360, 1432-1890, DOI 10.1007/s00572-014-0585-4, [Consultado: 14 de febrero de 2017], Disponible en: <http://link.springer.com/article/10.1007/s00572-014-0585-4>.
22. Hsiao, T. C. "Plant Responses to Water Stress". *Annual Review of Plant Physiology*, vol. 24, no. 1, 1973, pp. 519-570, ISSN 1543-5008, DOI 10.1146/annurev.pp.24.060173.002511, [Consultado: 14 de febrero de 2017], Disponible en: <http://dx.doi.org/10.1146/annurev.pp.24.060173.002511>.
23. Ben-Rejeb, K.; Abdelly, C. y Savouré, A. "How reactive oxygen species and proline face stress together". *Plant Physiology and Biochemistry*, vol. 80, 2014, pp. 278-284, ISSN 0981-9428, DOI 10.1016/j.plaphy.2014.04.007, [Consultado: 14 de febrero de 2017], Disponible en: <http://www.sciencedirect.com/science/article/pii/S0981942814001417>.
24. Bunnag, S. y Pongthai, P. "Selection of Rice (*Oryza sativa* L.) Cultivars Tolerant to Drought Stress at the Vegetative Stage under Field Conditions". *American Journal of Plant Sciences*, vol. 04, no. 09, 2013, pp. 1701-1708, ISSN 2158-2742, DOI 10.4236/ajps.2013.49207, [Consultado: 14 de febrero de 2017], Disponible en: <http://www.scirp.org/journal/PaperInformation.aspx?PaperID=36114&#abstract>.
25. Florido, B. M. y Bao, F. L. "Tolerancia a estrés por déficit hídrico en tomate (*Solanum lycopersicum* L.)". *Cultivos Tropicales*, vol. 35, no. 3, 2014, pp. 70-88, ISSN 0258-5936, [Consultado: 14 de febrero de 2017], Disponible en: <http://scielo.sld.cu/scielo.php?script=sci_abstract&pid=S0258-59362014000300008&lng=es&nrm=iso&tlng=es>.
26. Noctor, G.; Mhamdi, A.; Chaouch, S.; Han, Y.; Neukermans, J.; Marquez-Garcia, B.; Queval, G. y Foyer, C. H. "Glutathione in plants: an integrated overview". *Plant, Cell & Environment*, vol. 35, no. 2, 2012, pp. 454-484, ISSN 1365-3040, DOI 10.1111/j.1365-3040.2011.02400.x, [Consultado: 14 de febrero de 2017], Disponible en: <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-3040.2011.02400.x/full>.

27. Noctor, G.; Mhamdi, A. y Foyer, C. H. "The Roles of Reactive Oxygen Metabolism in Drought: Not So Cut and Dried". *Plant Physiology*, vol. 164, no. 4, 2014, pp. 1636-1648, ISSN 1532-2548, DOI 10.1104/pp.113.233478, [Consultado: 14 de febrero de 2017], Disponible en: <http://www.plantphysiol.org/content/164/4/1636>.
28. Lamz, P. A.; Reyes, G. Y. y González, C. M. C. "Indicadores bioquímicos para la selección temprana de genotipos de arroz (*Oryza sativa* L.) con tolerancia a la salinidad". *Cultivos Tropicales*, vol. 34, no. 1, 2013, pp. 11-17, ISSN 0258-5936, [Consultado: 14 de febrero de 2017], Disponible en: <http://scielo.sld.cu/scielo.php?script=sci_abstract&pid=S0258-59362013000100002&lng=es&nrm=iso&tlng=es>.
29. Çelik, Ö. y Atak, Ç. "The effect of salt stress on antioxidative enzymes and proline content of two Turkish tobacco varieties". *Turkish Journal of Biology*, vol. 36, no. 3, 2012, pp. 339-356, ISSN 1300-0152, [Consultado: 14 de febrero de 2017], Disponible en: <http://journals.tubitak.gov.tr/biology/abstract.htm?id=12700>.
30. Caverzan, A.; Passaia, G.; Barcellos, R. S.; Werner, R. C.; Lazzarotto, F. y Margis-Pinheiro, M. "Plant responses to stresses: role of ascorbate peroxidase in the antioxidant protection". *Genetics and Molecular Biology*, vol. 35, no. 4, 2012, pp. 1011-1019, ISSN 1415-4757, DOI 10.1590/S1415-47572012000600016, [Consultado: 14 de febrero de 2017], Disponible en: <http://www.scielo.br/scielo.php?script=sci_abstract&pid=S1415-47572012000600016&lng=en&nrm=iso&tlng=en>.
31. Aranjuelo, I.; Doustaly, F.; Cela, J.; Porcel, R.; Müller, M.; Aroca, R.; Munné-Bosch, S. y Bourguignon, J. "Glutathione and transpiration as key factors conditioning oxidative stress in *Arabidopsis thaliana* exposed to uranium". *Planta*, vol. 239, no. 4, 2014, pp. 817-830, ISSN 0032-0935, 1432-2048, DOI 10.1007/s00425-013-2014-x, [Consultado: 14 de febrero de 2017], Disponible en: <http://link.springer.com/article/10.1007/s00425-013-2014-x>.

Received: June 6th, 2016
Accepted: December 16th, 2016