ISSN impreso: 0258-5936
ISSN digital: 1819-4087

Ministerio de Educación Superior. Cuba
Instituto Nacional de Ciencias Agrícolas
http://ediciones.inca.edu.cu

EFFECT OF SIMULATED DROUGHT USING PEG–4000 ON GERMINATION AND SEEDLING GROWTH OF TWO RICE CULTIVARS (*Oryza sativa* L.)

Efecto de la sequía simulada con PEG–4000 sobre la germinación y el crecimiento de las plántulas de dos cultivares de arroz (*Oryza sativa* L.)

Elizabeth Cristo[1]✉, María C. González[1] and Elsa Ventura[2]

ABSTRACT. This work was made at Genetics lab from Technological Scientific Basic Unit "Los Palacios" (UCTB), belonging to the National Institute of Agricultural Sciences (INCA), located in Los Palacios, Pinar del Río province. The aim was to evaluate the effect of simulated drought with PEG-4000 on germination and seedling growth of two rice cultivars seeds. Selected cultivars were Guillemar LP-19 and Perla de Cuba (as tolerant control). They were treated at different concentrations of osmotic agent (PEG 15, 20, 25, 30 g L^{-1}) and a control treatment with distilled water was also put. It was evaluated the germination percentage, plant height, the root system length and their dry mass. It was shown differential behaviours among cultivars under drought levels caused by different PEG concentrations. The cultivar "Guillemar LP–19" showed the better behaviour for the four analyzed parameters than tolerant control cultivar. First stages of plant growth presented affectations with the increasing of the PEG-4000 concentrations, as osmotic agent to simulate the stress for drought.

Key words: low water supplies, cultivars, abiotic stress

RESUMEN. El trabajo se desarrolló en el laboratorio de genética de la Unidad Científico Tecnológica de Base "Los Palacios" (UCTB), perteneciente al Instituto Nacional de Ciencias Agrícolas (INCA), ubicado en el municipio Los Palacios, provincia Pinar del Río. El objetivo fue evaluar el efecto de la sequía simulada con PEG–4000 sobre la germinación y el crecimiento de las plántulas de arroz. Se seleccionaron semillas de dos cultivares Guillemar LP–19, y Perla de Cuba (control tolerante a la sequía) a diferentes concentraciones del agente osmótico (PEG 15, 20, 25, 30 g L^{-1}) y un control con agua destilada. Se evaluó el porcentaje de germinación, altura de las plantas, la longitud del sistema radical y la masa seca, mostrándose un comportamiento diferenciado en los cultivares bajo diferentes concentraciones de PEG. El cultivar Guillemar LP–19 mostró el mejor comportamiento, en los cuatros indicadores analizados para este tipo de estrés abiótico, en comparación al testigo tolerante. Con el aumento de las concentraciones de PEG-4000 como agente osmótico para simular el estrés por sequía, los primeros estadios de crecimiento de las plantas presentaron afectaciones.

Palabras clave: bajos suministros de agua, cultivar, estrés abiótico

INTRODUCTION

Rice cultivation (*Oryza sativa* L), began to be cultivated almost 10,000 years ago, in many humid regions of tropical and subtropical Asia. Currently, this cereal is the staple food for more than half of the world's population. It occupies second place after wheat with respect to harvested surface (1). Rice provides more calories per hectare than any of the other cultivated cereals (1).

In Cuba it constitutes the main food for the population with an annual consumption close to 60 kg per capita. It is among the first places in Latin America; but until now, the national production only satisfies a little more than 50 % of the needs, so the country is forced to complete them with imports (2,3).

In the country, the current commercial cultivars have shown a potential of yield that exceeds 7 t ha^{-1}; In spite of this, the productive conditions in the last 20 years do not exceed 3,5 t ha^{-1} as average (3,4), caused by different causes, highlighting the low

[1] Instituto Nacional de Ciencias Agrícolas, Gaveta postal No.1, San José de las Lajas. Mayabeque, Cuba. CP 32700

[2] Centro de Desarrollo de Productos Bióticos del Instituto Politécnico Nacional, carretera Yautepec-Jojutla km 8, Calle CEPROBI, No. 8, Col. San Isidro, Yautepec, Morelos, México. CP 62731

✉ ecristo@inca.edu.cu

availability of water. The drought together with the salinity of the soils is a serious problem, which affects the yield of crops and the sustainability of agriculture; About 10 % of the planet's surface is affected and some 10 million hectares are abandoned due to that (5). In Cuba, drought has affected about 76 % of the cultivated areas, mainly in the eastern provinces, and it is stated that it is the abiotic stress that has the greatest impact on the growth and development of plants (4) and, specifically, one of the factors that limit the productivity of rice (6-9). Plants, in response to drought, have developed mechanisms to maintain a favorable water concentration, either by conserving the water or by its ability to provide it to the aerial part, even in deficit conditions (6,10).

Such situation has motivated that the works directed to the recovery and the handling of these soils have intensified in the last years (8), and programs of genetic improvement are developed directed to the obtaining of tolerant cultivars to the drought, with the purpose of increase yields and recover, in addition, areas with little water availability (4,5,8).

For the development of the breeding program, the detection of markers that can be used to identify tolerant genotypes in early stages of development is indispensable. Studies carried out with rice cultivation have suggested the possibility of using some simple indicators, such as the percentage of germination and early growth for the tolerance of the materials obtained for environmental stress (5,11).

Therefore, the objective of this work was to evaluate the effect of simulated drought with PEG-4000 on the germination and growth of seedlings in two rice cultivars, using agronomic morph markers for the early selection of promising genotypes.

MATERIALS AND METHODS

The work was developed in March 2013, under controlled conditions, in the Genetics Laboratory of the Scientific Technological Unit of the "Los Palacios" Base. 100 seeds were selected from two rice cultivars "Guillemar LP-19" and Perla de Cuba (Table I), the latter as drought tolerance control, which were placed to germinate in Petri dishes with filter paper, and moistened with 15 mL of Polyethylene glycol solution (PEG-4000), at 15, 20, 25, 30 g L^{-1} to simulate the stress of drought. As a control, distilled water was used. The plates were placed in a growth chamber, at a temperature of 28 ± 2 ˚C. The number of germinated seeds was evaluated seven days later, the germination percentage was determined and after 15 days the height of the seedlings, root length and accumulated dry biomass in ten seedlings per replica were evaluated. A completely randomized design with three replicates was used and the data were analyzed statistically using a confidence interval for $\alpha \leq 0.05$.

Table I. Genotypes evaluated in the study and their origin

No	Genotypes	Progenitors	Origin
1	Guillemar LP-19	Amistad–82/INCA LP–7	Cuba
2	Perla de Cuba	Unknown	Cuba

RESULTS AND DISCUSSION

It was found that although differences were observed between the treatments studied, there is a reduction in the germination percentages in the two genotypes evaluated (Figure 1), as the PEG concentrations increased, being statistically higher from the concentration of 15 g L^{-1}. This decrease in germination can be attributed to the fact that high concentrations of PEG-4000 prevent the absorption of water by the seeds, due to the low osmotic potential that is created in the solution since it reduces the availability of oxygen, by limiting its solubility and diffusion, given its high viscosity (8,9). In studies carried out by other authors in crops such as wheat, beans and corn, it obtained the same behavior (9,12).

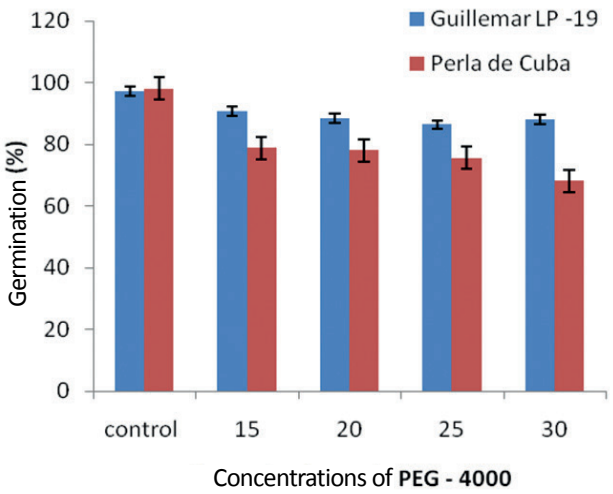

Figure 1. Influence of PEG-4000 concentrations on seed germination of two rice cultivars

When evaluating the growth, differential inhibitions were detected in the final height, root length and dry mass of the seedlings in the cultivar "Perla de Cuba", in comparison with the control (Figure 2 and 3), but the damages were lower in the cultivar "Guillemar LP-19" at the concentration of 15 g L^{-1}. Such affectations in growth coincide with what is observed in this culture (4, 13-16). In this regard, it points out that the detrimental effect of PEG-4000 could be explained by the water deficit that it produces in the growing foliar tissues, by decreasing the turgor of the cells and changes in the permeability of the membranes. Other researchers found that the length of the main root increases in conditions of water stress, because the plant needs to drink water in the area with the highest humidity and continue its physiological processes.

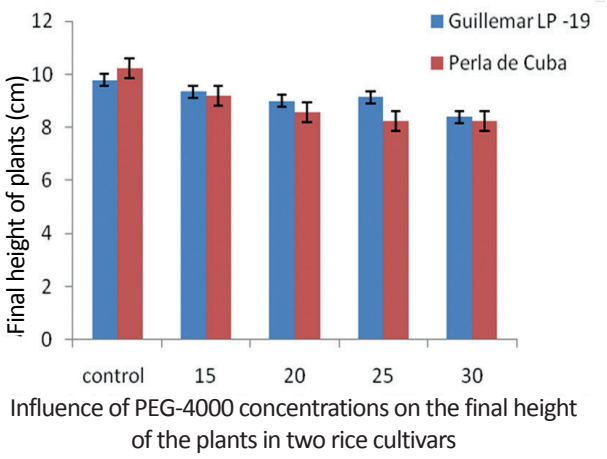

Figure 2. Influence of PEG-4000 concentrations on the final height of the plants in two rice cultivars

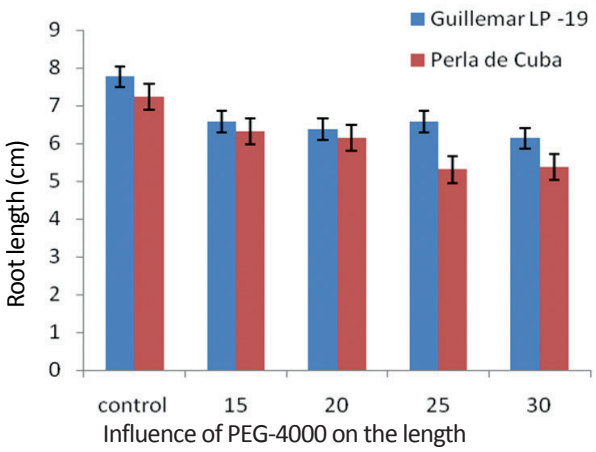

Figure 3. Influence of PEG-4000 concentrations on the length of the root in two rice cultivars

The differences found in the cultivars in terms of germination and seedling growth indicate the usefulness of these morphological agronomic indicators in the early selection of drought tolerant genotypes. On the other hand, some researchers point out that the additive variance for the percentage of germination, height and root length of the seedlings grown in PEG-4000 solutions was much higher than the dominant variance, showing the efficiency of their use in the segregant generations (13).

This aspect is of great importance, since the successes of the tolerance evaluation to drought in the field are low, laborious and provide very variable results (12), so laboratory methods are a valid option to discriminate genotypes in early stages of breeding programs. In this regard, it has been indicated that one of the most commonly used methods to determine the tolerance of plants to water stress is the evaluation of the germinative capacity of the seeds in these stressful conditions (4,6-8). On the other hand, it has been pointed out that in order to simulate the conditions of drought under laboratory conditions and to study the processes of germination and growth of plants, aqueous solutions of mannitol and PEG have been used, as they are inert and non-toxic compounds plants (7,9-11).

Regarding the dry mass of the seedlings (Figure 4), differences were found compared to the control, from concentrations of 20 g L -1 in the two cultivars, showing a decrease in the cultivar "Perla de Cuba" while the cultivar "Guillemar LP-19" presented the least affectation, showing an acceptable behavior of the dry mass of the plant since it presents a more accelerated growth than the Pearl of Cuba, which is a drought tolerant cultivar. The "Guillemar LP-19" differs from the tolerant control in the concentrations of 25 and 30 of PEG which could be attributed among other causes that as the PEG concentrations increase, the height of the plants is affected, this corroborates the results presented by several authors who indicated that as the concentrations of PEG-4000 increase, the growth of the seedlings caused by both the effect of osmotic stress and by the imbalance of specific ions and toxic effects caused by the excessive accumulation of ions decreases. (4).

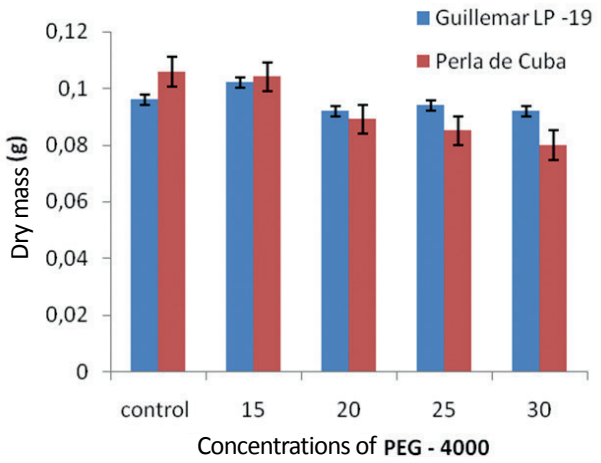

Figure 4. Influence of PEG-4000 concentrations on the dry mass in two rice cultivars

CONCLUSIONS

- It can conclude that the water deficit constitutes one of the abiotic factors limiting the productivity of rice. The low availability of water causes different effects on the physiological processes in plants, which vary between species and cultivars of the same species and depends on the level of severity of the deficit and the plant physiological state, which was achieved with the increase in PEG concentrations.
- It is a subject of great importance, taking into account that currently there is a high percentage of areas that are dedicated to this cereal that cannot be sown due to lack of stable supply of irrigation water and suitable cultivars for it.
- We consider that another experiment should be designed and these same cultivars evaluated with PEG-6000 with higher osmotic potential concentrations, to evaluate if the difference is more marked.

BIBLIOGRAPHY

1. Jiménez O, Silva R, Cruz J. Efecto de densidades de siembra sobre el rendimiento en el arroz (*Orysa sativa* L.) en el Municipio Santa Rosalia, Estado de Portuguesa, Venezuela. Revista Unell Cienc Tec. 2009;27:32-41.
2. Polón R, Castro R , Ruiz M, Maqueira LLA. Practicas de diferentes alturas de corte a cultivo de rebrote y su influencia en el rendimiento del arroz (*Oryza sativa* L.) en una variedad de ciclo medio. Cultivos Tropicales. 2012;33(4):59-62.
3. Instituto de Investigaciones del Arroz. Instructivo Técnico del Arroz. La Habana, Cuba; 2014. 73 p.
4. García A, Dorado M, Pérez I, Montilla E. Efecto del déficit hídrico en la distribución de fotoasimilados en plantas de arroz (*Oryza sativa* L.). Interciencia. 2010;35(1):46-54.
5. Cristo VE, González MC, Pérez NJ. LP – 20 Nuevo cultivar de arroz (*Oryza sativa* L.) tolerante a los bajos suministros de agua y fertilizantes. Cultivos Tropicales. 2015;36(2):95.
6. Pandey V, Shukla A. Acclimation and tolerance strategies of rice under drought stress. Rice Science. 2015;22(4):147-61.
7. Silmara CF, Juliano SC. Processo germinativo de sementes de Paneira sob estresses hídrico e salino. Pesquisa Agropecuaria Brasileira. 2004;39(9):24-31.
8. Swain P, Anumalla M, Prusty M, Marndi BC, Rao GJN. Characterization of some Indian native land race rice accessions for drought tolerance at seedling stage. Journal Crop Sci. 2014;8(3):324–31.
9. Heidary RY, Heidary M. Evaluation of resistance for salinity drought cold an pH change in four Iranian wheat cultivars. Journal of Agricultural Science and Natural Resources. 2002;9(1):32-8.
10. Ojeda S, Michel C, Amador MB, Escobar IM, Diéguez T, Ruiz EE, Higinio F, Nieto GA. Estrés hídrico en la germinación y crecimiento de plántulas de genotipos de albahaca *Ocimum basilicum* L. Revista Mexicana de Ciencias Agrícolas. 2013;4(2):229-41.
11. Ogbonnaya CI, Sarr B, Brou C, Diouf O, Diop NN, roy–Macauley H. Selection of cowpea genotypes in hydroponics pots and field for drought tolerance. Crop Science. 2003;43:1114–20.
12. Domínguez SA. Efecto del estrés hídrico sobre la germinación de genotipos de frijol común en condiciones experimentales de sequía. Revista Avanzada Científica. 2014;17(1):1-15.
13. Bunnag S, 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. 2013;4(9):1701-8.
14. Sokoto MB, Muhammad A. Response of rice varieties to water stress in Sokoto, Sundan Savannah, Nigeria. J Biosciences Medicines. 2014;2(1):68–74.
15. García MS, Gómez M, Libia IF, Téllez T, Herrera CÉ. Factores de transcripción involucrados en respuestas moleculares de las plantas al estrés osmótico. Revista Fitotecnia Mexicana. 2013;36(2):105–15.
16. Dalpat LHE, Shashidhar PH, Ramanjini G, Ashok TH. Department Callus Induction and Regeneration from in vitro anther Culture of Rice (*Oryza sativa* L.). International Journal of Agriculture Environment & Biotechnology. 2014;7(2):213-8.

Received: June 29th, 2016
Accepted: December 26th, 2016