Original Article

INFLUENCE OF HUMIC SUBSTANCES ON THE PERFORMANCE AGRONOMIC ANALYSIS OF TWO RICE LINES SUBJECTED TO DIFFERENT SALINITY LEVELS AT GERMINATION AND EARLY GROWTH STAGES

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Abstract

The objective of this study was to evaluate the effect of humic substances on the tolerance of two rice varieties to salt stress, as well as to know the main characteristics of the scientific production registered in the Scopus database regarding the publication of research related to the variables Humic Substances, Agronomic Behavior of Rice, Germination Stage, and Initial Growth Stage. The experiment was carried out in a farmhouse at the Faculty of Agricultural Sciences of the UPSE, La Libertad-Santa Elena. Two rice varieties were used, INIAP FL-Arenillas and INIAP FL-1480, with medium salinity tolerance. The seeds were established in plastic containers containing three levels of salinity (0, 75 and 150 mM NaCl) and applying three concentrations of humic substances from vermicompost (0, 5 and 10 mg C L-1). Two phases were established. In the first phase, which lasted seven days, germination parameters (IVG, Vigor and %G) were evaluated, and in the second phase, initial growth (dry biomass and height) was evaluated at zero and five days after seed germination. Hoagland´s nutrient solution was used at half strength in the second phase, which was replenished daily. A tri-factorial DCA experimental design (3x3x2) was used, in which the following factors were evaluated: salt stress (ES), humic substances (SH) and variety (V), and the interaction between them, with a total of 54 experimental units divided into 18 treatments and three replications. The data obtained were statistically processed by the SISVAR 2019 program. The results were as follows: the FL-1480 variety presented greater resistance to the different levels of salinity applied to FL-Arenillas, the germination percentages of FL-1480 were higher by 9.78%, 19.56% and 22.22% when compared to FL-Arenillas for ES levels of 0, 75 and 150 mM NaCl respectively, the IVG and vigor presented similar behavior. It was

observed that the application of SH reduced the percentage of abnormal seedlings significantly for the variety FL-Arenillas for all treatments under salt stress; similar results were observed for the variety FL-1480, although without significant results. In the case of FL-1480, doses of 5 and 10 mg increased the root growth of plants under ES levels of 150 mM NaCl. The FL-Arenillas variety showed a stimulating effect on root growth when doses of 5 mg C L-1 were applied to seedlings under salt stress levels of 75 and 150 mM NaCl. Applying humic substances from vermicompost to the two varieties reduced the number of abnormal seedlings obtained due to salt stress and increased total dry biomass and root length. Each variety had a specific SH dose for root length, the application of 5 mg C L-1 for FL-Arenillas at both ES levels, 5 and 10 mg C L-1 in FL-1480 for severe ES levels.
Keywords: Humic Substances, Agronomic Performance of Rice, Germination Stage, Initial Growth Stage.

1. Introduction

Salinity is a problem that has affected humanity since its beginnings, and its origin is related to natural and anthropic factors. For example, salinity is one of the limiting factors of agricultural production in Ecuador, causing losses of between 4% and 6% (Poveda and Andrade, 2018).

In Ecuador, rice cultivation is one of the most extensive, with the coastal region accounting for more than a third of the transitional crop area, making it one of the most important crops in the country. According to Mendoza (2019), 29 million tons of rice production in 2017 came from Latin American countries, placing Ecuador in the No. 26 position as an exporter.

Although conditions for rice cultivation are favorable in most rice-growing areas, it has been reported that 8% of the soils in the provinces where rice is grown (Manabí, Guayas and El Oro) have some salinity (Hernández et al., 2012).

The rice crop tolerates flooding conditions, and its tolerance to salinity is low, affecting the yield. The main effects of salinity on the rice crop are related to physiological and metabolic damage, related to the alteration of homeostatic processes, which result in reduced germination capacity, reduction of leaf and radicle area and reduction in biomass production (Mena, 2016; Tapia et al., 2022).

This situation highlights the need for viable alternatives to cope with these conditions, among them the use of salt stress tolerant varieties such as Jucarito-104 in Cuba (Hernández et al., 2018) and the interspecific cross Puyon/JP002 P8-32 P35 I:20 in Ecuador (Sánchez, 2020), and the application of humic substances as one of the ecologically and economically viable alternatives (Morocho, 2014), since others such as the washing of soil salts do not apply to regions with water resource use restrictions.

The positive physiological effect of using humic substances in saline conditions has been studied in crops such as beans (Canellas et al., 2015), in which it reduced membrane permeability and in tomatoes (Hernández et al., 2016), in which it improved fruit quality.

In the last decades, several studies have revealed that humic substances from vermicompost have a biostimulant effect on plant metabolism and physiology (Veobides et al., 2018), which is reflected in the increased growth of rice (Guridi-Izquierdo et al., 2017), tomato (Torres et al., 2016) and maize (Pinos et al., 2019) crops, agreeing that their use stimulated the growth of stems, roots, leaves, and fruits.

The objective of this study was to evaluate the influence of humic substances on the agronomic performance of two rice varieties subjected to different salinity levels during the germination and initial growth stages.

2. General Objective

To evaluate the influence of humic substances on the agronomic performance of two rice varieties subjected to different salinity levels during the germination and initial growth stages.

3. Methodology

3.1 Materials and methods

Two rice varieties were used, with a medium level of tolerance to salinity: INIAP FL-Arenilllas and INIAP FL-1480, which were subjected to salinity levels of 0.75 and 150 mM NaCl under the influence of three concentrations of humic substances of 0, 5 and 10 mg C L-1. A 2x3x3 trifactorial DCA experimental design was used, with 18 treatments and three replications per treatment for 54 experimental units. Each experimental unit consisted of a polypropylene cup containing 25 seeds each. The SH was extracted from bovine manure vermicompost and characterized by spectrometric techniques in EMBRAPA-Agrobiology laboratories and the Federal Rural University of Rio de Janeiro by Pinos et al. (2019).

3.1.1 Germination

The previously disinfected seeds were placed to germinate in polypropylene cups prepared to support the seeds containing the treatments and evaluated daily until day seven to determine the IVG according to the Maguire methodology (1962), on the last day of evaluation, vigor and germination percentage were determined according to Labouriau (1976).

3.1.2 Initial growth

Once the rice seeds were germinated, seedlings considered normal and with morphological and homogeneous characteristics of each treatment were chosen to continue the experiment. These seedlings were evaluated on the day of installation of the experiment and five days after germination. During the evaluation, the following variables were determined: aerial part length (cm), root length (cm), and dry biomass (mg). The results obtained were subjected to analysis of variance by F-test by the statistical program Sisvar (Ferreira, 2019; Franco et al., 2022). In addition, the Tukey test was applied at 0.05% significance when the effects were significant. Graphs were processed with Microsoft Excel 365 software.

3.2 Bibliometric review

In order to know the current status of the bibliography published through high-impact journals indexed in the Scopus database, a bibliometric review is carried out in the same database on research papers concerning the study of Humic Substances, Agronomic Behavior of Rice, Germination Stage, Initial Growth Stage and published during the period between the years 2017 and 2021. As a result, the identification of 186 papers published globally indicates certain characteristics in terms of production volume. This article explains the Co-occurrence of Keywords used in these documents, the Country of Origin of the publication and the Area of Knowledge in which the published research was based.

3.2.1 Word co-occurrence

The following figure shows the correlation between variables used in the research taken as a reference.

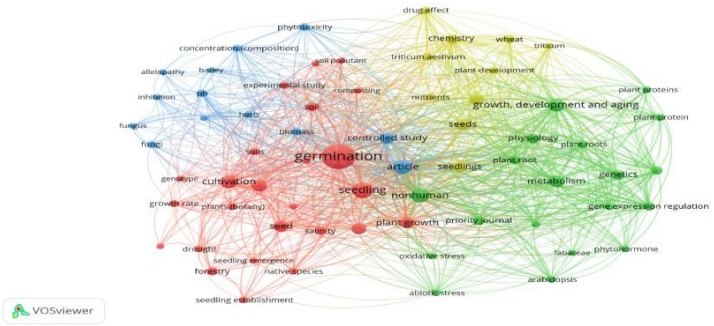

Figure 1. Co-occurrence of keywords

Source: Propis (2022); based on data provided by Scopus.

Germination was the key word most frequently used in the research identified, associated with variables such as Cultivation, Seedlings, Salinity, Silviculture, and Soil Contamination, among others that represent ideal conditions for the germination of agricultural products such as rice. On the other hand, Growth, Development and Aging lead to the scientific production of a subset of publications related to Physiology, Regulation of Gene Expression, Metabolism, Plant Rices, and Plant Protein, which allows inferring that these publications have good theoretical bases for the construction of new knowledge around the mechanisms created to support plant growth by genetic means as shown according to the keywords used and their correlation with the general context of the bibliometric data.

3.2.2 Distribution of scientific production by country of origin

The distribution of the scientific production identified in Scopus, according to the country of origin of the publication, is shown below.

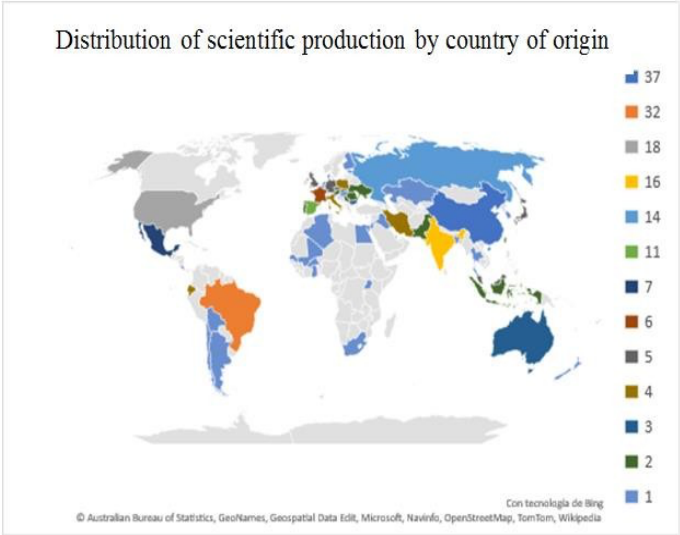

Figure 2. Distribution of scientific production by country of origin

Source: Prepared by Propis (2022); based on data provided by Scopus.

According to Scopus, China had the highest number of records during 2017-2021, with 37 publications in high-impact journals indexed in that platform, followed by Brazil with 32. It is important to highlight how the two countries that lead the scientific production at a global level, referring to the study of the influence of humic substances on the agronomic behavior of rice, focused on its germination and growth, are traditional from countries with a large volume of the annual production of this cereal, so it can be inferred that the research associated with this topic, arouses great interest based on the needs that have been identified through the cultivation of this and many other products. It is important to note that each publication may have the participation of two or more authors affiliated with different institutions that are not even in the same country, so this document is counted as a unit for each participating country.

3.2.3 Distribution of scientific production by area of knowledge

Figure 3 shows how scientific production is distributed according to the area of knowledge through which the scientific papers were developed.

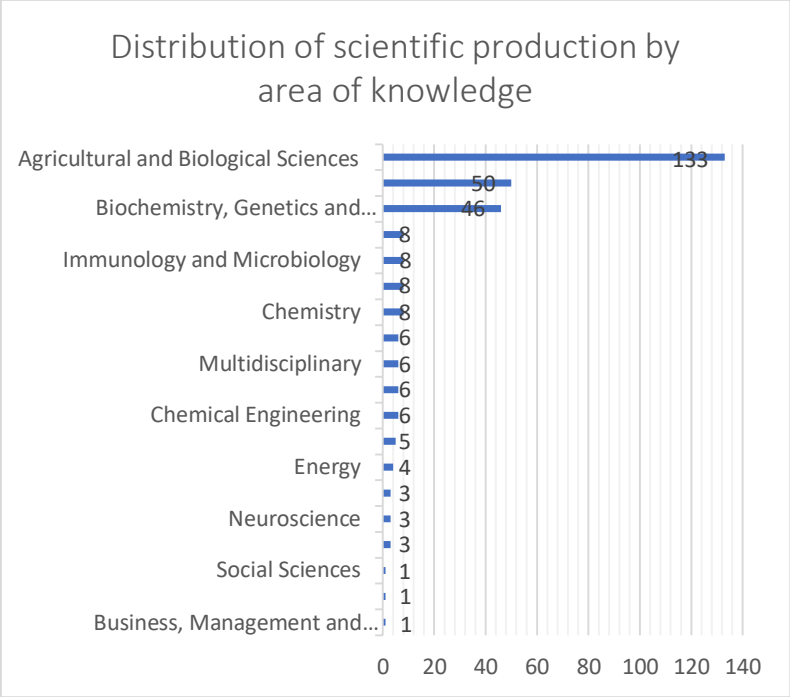

Figure 3. Distribution of scientific production by area of knowledge.
Source: Own elaboration (2022); based on data provided by Scopus.

The area of Agriculture and Biological Sciences proved to be the theoretical basis on which most of the publications were based to carry out their methodologies, and this is due precisely to the nature of the

variables, which have a great influence on the development of techniques in the practice of rice cultivation as the object of study of this article. 133 publications were based on applying theories derived from this area. Environmental Sciences was in second place with 50 papers, and Biochemistry, Genetics and Molecular Biology were in third place with 46 research papers dealing with this topic as proposed.

4. Results and discussions

4.1 Germination

Significant differences were detected in the factors variety and salt stress in the variables germination, IVG and vigor; significance was observed in the interaction variety V x ES in germination and percentage of normal seedlings. In addition, the variable SH was significant for the variable percentage of abnormal seedlings.

Variety FL-1480 presented a higher germination percentage concerning FLArenillas under the three NaCl concentrations, which in percentage terms represents a reduction of 9.78%, 19.56% and 22.22% in germination percentage under the same salt stress conditions as can be seen in Table 1. Therefore, variety FL-1480 was not significantly affected by the increase in NaCl concentration.

Varieties	Germination (%)		
	0	mM NaCl	
FL-Brushes 89.33	bA	79.55 bB	69.33 bC
FL-1480	99.11 aA	99.11 aA	91.55 aB

Table 1. Response of the interaction of the variety of the factors x salt stress on the germination percentage of two rice varieties subjected to salt stress and treated with humic substances.

Means followed by the same letter do not differ statistically; lower case letters compare means between rows and upper case letters compare means between columns, according to Tukey's test ($p<0.05$).

Table 3 shows the effects of different salinity levels on two rice varieties and the application of three doses of SH. In general, variety FL-1480 showed a lower percentage of abnormal seedlings than FL-Arenillas. However, it was observed that when the seeds of the FL-Arenillas variety were exposed to salinity levels of 75 and 150 mM NaCl, the percentage of abnormal seedlings increased by 1.33% and 13.27% compared to the control without SH and NaCl, an effect that was significantly reduced when SH doses of 5 and 10 mg C L-1 were applied by 12 and 16% concerning the treatment without SH and with 150 mM NaCl.

Salt stress (mM NaCl)	Abnormal seedlings (%)					
	FL-Brushes			FL-1480		
	Humic substances (mg C L^{-1})					
	SH-0	SH-5	SH-10	SH-0	SH-5	SH-10

0	5,33	b A	1,33	aA	1,33	aA	1,33	aA	1,33	aA	0.00 aA
	6,66	b A	5,33	aA	2,66	aA	5,33	aA	4,00	aA	1.33 aA
	18,66	aA	6,66	aB	2,66	aB	2,66	aA	0,00	aA	5.33 aA

Table 2. Response of the interaction of the factors V x ES x SH on the percentage of normal seedlings (NP) and abnormal seedlings (AP) of two rice varieties subjected to salt stress and treated with humic substances.
Note: Means followed by the same letter do not differ statistically; lower case letters compare means between rows and upper case letters compare means between columns, according to Tukey's test ($p<0.05$).

Similar results were found (Solórzano, 2019) in bell pepper and by (Reyes et al., 2014) in Albaca, indicating that germination processes are drastically affected in the presence of ES due to the harmful effects caused at the physiological level being the application of humic substances a viable solution that influences the increase of germination % and IVG under these conditions, furthermore (PÉREZ, 2010) indicates that SH reduces the number of abnormal seedlings due to the induction of protein and enzymatic activation and defense mechanisms against abiotic stress in crops.

4.2 Initial growth

When analyzing the interaction between the factors SH x V x ES, a significant reduction in root length was observed as the NaCl concentration increased for the two varieties. Seedlings subjected to ES levels of 150 mM NaCl and under the effect of SH doses of 5 and 10 mg C L-1 presented an increase in root length concerning the treatment without application of SH for variety FL-1480, presenting increases of 2.05 and 2.35 cm concerning the treatment with ES and without SH.

In the case of the FL-Arenilla variety, the application of doses of 5 mg C L-1 of SH had a stimulating effect on root growth in the treatments under salt stress of 75 and 150 mM NaCl, with increases of 0.87 cm and 2.2 cm, respectively.

	FL-1480						**FL-Arenilla**					
	SH mg C L-1											
		aA		aA		aA		aA		aA		aA
EN							**0**					
75	1	aA	8,81	bA	9,76	bA	6,76	bA	7,63	bA	4,76	bA
	3,91	bA	5,96	bA	6,26	cA	2,76	cA	4,96	bA	2,73	bA

The dry biomass variable presented statistical significance in the ES and SH factors independently (Figure 2), indicating that as NaCl concentrations increase, seedling weight is significantly reduced by 4 and 7.9 mg in concentrations of 75 and 150 Mm of NaCl about the control treatment without ES , and that the application 5 mg C L-1 of SH would be the best dose for this variable increasing the weight by 0.8 mg about the control without SH, coinciding with (LASTIRI et al..., 2017) and (REYES-PÉREZ et al., 2014). In addition, they found that the increase of salts reduces fresh and dry weight in seedlings however the application of SH would increase it.

Conclusions

The application of humic substances from vermicompost acted in the germination phase reducing the number of abnormal seedlings obtained due to salt stress in both varieties. However, FL-Arenillas were the most affected by salinity. At the same time, in the initial growth stage, the total dry biomass and root length also increased with the difference that for the second variable mentioned, each variety had a dose of SH, in which it showed better characteristics being 5 mg C L-1 for FL-Arenillas in both levels of ES, 5 and 10 mg C L-1 in FL-1480 for severe levels of ES.

It is hoped to encourage research on this type of study since, through the analysis of current theories and practices, it is possible to learn about their weaknesses and strengths, as well as opportunities for improvement and reengineering that will allow the design of new and better tools and techniques for the practice of rice agriculture and other products.

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