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ESCALA CUANTITATIVAPARA MEDICIÓN DEL GRADO DE INFECCIÓN EN SEMILLAS DE ARROZ A NIVEL DE LABORATORIO

QUANTITATIVE SCALE FOR MEASUREMENT OF INFECTION GRADE IN RICE SEEDS AT LABORATORY LEVEL

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RESUMEN

En la investigación se reactivaron los aislados bacterianos en medio líquido JMV para evaluar la virulencia de *Burkholderia glumae* frente a semillas de arroz certificadas por Fedearroz variedades F67, F68 y F2000; se procedieron a desinfectar las semillas, consecutivamente se dejaron en agua destilada estéril por 2 días; luego se secaron y se ajustó el DO del inóculo de *Burkholderia glumae* (aislados Bg007, Bg010 y Bg011), realizando diluciones para evaluar concentraciones desde 10^8 hasta 10^3 células por mililitro; se procedió a agitar las semillas con el inóculo, llevadas posteriormente vacío durante 10 minutos por cada dilución llevándose a cajas de Petri con papel filtro humedecido con agua destilada estéril durante 7 días; crecidas las plántulas se procedieron a medir parámetros y nivel de severidad. Se procedió a hacer el análisis estadístico mediante Software es IBM SPSS Statistics, el cual permitió obtener Gráficas De Dispersión, Diagramas De Barras De Error; Comparaciones Multivariante, la Prueba de Muestras Relacionadas se realizó a partir Comparación de las Medias. Se hizo conteo de frecuencias, junto con

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sus Diagramas de Frecuencias, se dividió las medidas en quintiles para realizar una escala cuantitativa de severidad. El análisis estadístico permitió evidenciar que la cepa más virulenta fue el aislado Bg007 y la menos virulenta el aislado Bg011, la variedad de arroz más afectada fue la F2000 y la menos afectada fue la F68.

PALABRAS CLAVE: Severidad, aislados bacterianos, análisis estadístico, virulencia**.**

ABSTRACT.

In the research, the virulence of *Burkholderia glumae* against rice seeds certified by Fedearroz varieties F67, F68 and F2000 was evaluated; the bacterial isolates were reactivated in liquid medium JMV; the seeds were disinfected, then left in sterile distilled water for 2 days; then the DO of the *Burkholderia glumae* inoculum (isolated Bg007, Bg010 and Bg011) was dried and adjusted, making dilutions to evaluate concentrations from 10^8 to 10^3 cells per millilitre; the seeds were stirred with the inoculum, then taken empty for 10 minutes for each dilution and placed in Petri boxes with filter paper moistened with sterile distilled water for 7 days; when the seedlings were flooded, the parameters and severity level were measured. statistical analysis allowed to obtain dispersion graphs, error bar diagrams; multivariate Comparisons; the Test of Related Samples was performed from comparison of means, measurements were divided into quintiles to make a quantitative scale of severity. The statistical analysis showed that the most virulent strain was the Bg007 isolate and the least virulent was the Bg011 isolate, the rice variety most affected was the F2000 and the least affected was the F68.

KEY WORDS: Severity, bacterial isolates, statistical analysis, virulence.

INTRODUCTION

The cultivation of rice (*Oryza sativa*) is not free of phytosanitary problems: insects, weeds, microorganisms, among others; a very common disease in Colombian rice fields is produced by *Burkholderia glumae*, a Gram-negative soil microorganism, was discovered in 1967 under Pseudomonas *glumae* (Kurita y Tabei, 1967), then renamed to its present one in 1992 (Yabuuchi *et al.,* 1992); causes bacterial mildew or vanity of rice, bacteria causes withering of seed and panicle, pod rot; survives in leaves, pods, soil and seeds, spreads as the plant grows. Infection of the panicles occurs in the flowering of the rice plant; infected panicles can infect healthy panicles. *Burkholderia glumae* was reported in 1989 in Colombia, but only in 2007 were economic damages caused (Perez y Saavedra, 2011). In Colombia, if the infection with *Burkholderia glumae* is not controlled in time, it can cause losses between 80 to 90% in the rice crop, affecting the yield, the weight of the grain, the sterility of the flowers and the germination of the seed (Galvis y Carrillo, 2015). In some rice-growing areas of Colombia it is absent and in other rice-growing areas of Colombia it is present, as is the case of Norte de Santander. For this reason, the degree of infection of *Burkholderia glumae* isolates in three certified rice seed varieties was evaluated at the laboratory level in order to generate a quantitative scale for its measurement.

METHODOLOGY

This study was framed within a quantitative approach (Vergel-Ortega *et al.,* 2016), **the phase included reactivation of the isolates** where the previously selected isolates, obtained from rice farms located in the El Zulia Irrigation District, Norte de Santander, in February 2018, had been identified by sequencing presented a similarity of 99% with *Burkholderia glumae*, found in cryopreservation coded as Bg007, Bg010 and

Bg011 were reactivated in JMV broth at 28 º C±2 º C for 24-48 hours, after observing turbidity in the medium was made the sowing in the culture medium King B and incubated at 28 º C±2 º C for 24-48 hours for subsequent inoculation in seeds.

In third **phase obtaining the bacterial inoculum**, from the growing boxes, an OD adjusted inoculum of 0.5 to 605nm absorbance was prepared in Genesys 10S UV-VIS Thermo Scientific spectophotometer, corresponding to a concentration of 10^8 cell/mL, as reported by (Vergel-Ortega *et al.,* 2016) and (Moreno *et al*., 2013). From this inoculum, serial dilutions up to 10^3 were made in 9mL vials containing sterile peptoned water, using only sterile peptone water as the target. To disinfection of rice seeds, the seeds were immersed in 70% ethanol with agitation for 6 minutes, then 3 washes were made with sterile distilled water, the water was extracted and a 6% sodium hypochlorite solution was added for 6 minutes, 3 washes are made again with sterile distilled water. Finally, **the disinfected seeds** are left in sterile distilled water for 2 days to induce the pregermination process (Flórez y Uribe, 2011).

In the inoculation of rice seeds, after pregermination, the seeds are taken to Petri boxes with absorbent paper until dry. For the inoculation process concentrations ranging from 10^8 to 10^3 cells per milliliter were used. 30 seeds were added in each inoculum concentration; 30 minutes were left in agitation at 150 rpm in incubator with 311DS Labnet agitation; after this time vacuum was applied at a pressure of 90Kpa for 10 minutes with Rocker 400 vacuum pump. At the end of this procedure, the seeds were placed in sterile Petri boxes containing filter paper, placing 10 seeds per box in three repetitions for each concentration, 20mL of sterile distilled water were added to maintain a humid atmosphere and incubated at a temperature of 28ºC-32ºC for 7 days (Méndez *et al*., 2020).

EVALUATION OF THE SEVERITY OF THE INFECTION.

The qualitative scale of severity proposed by (Devescovi *et al.,* 2007) and (Flórez y Uribe, 2011) was taken as a reference. The following parameters were taken into account to measure: stem length, stem diameter, main root length, total plant length, number of roots, number of leaves.

STATISTICAL ANALYSIS.

The data obtained from the experimental design were evaluated using IBM SPSS Statistics Software, which allows obtaining error diagrams; diagram and frequency tables, quintiles. Taking into account the most significant variables in data collection from the relationship between inoculum concentration and severity of infection.

RESULTS

After reactivation of the isolates, typical morphological characteristics of *Burkholderia glumae* were observed, such as formation of greenish-yellow pigment in King B culture medium, small, creamy colonies with smooth edges and colonies with lens elevation. According to (Pedraza, 2012) purity and viability were also confirmed to continue with the study. After inoculation of these isolates in the seeds, different levels of severity according to the scale of (Vergel Ortega *et al.,* 2016) were evidenced in the three varieties F68, F69 and F2000. As the concentration of the inoculum increased, an inverse proportional relationship with germination was found in almost all the samples and differences in the damage caused by the bacteria in response to the concentrations of the pathogen (103 to 108). The Bg007 isolate showed to be the most severe strain for the 3 varieties evaluated while the Bg011 isolate showed the least severity. Variety F2000 was the variety most affected by the three bacterial

isolates evaluated and variety F68 was the least affected.

Plant resistance can be based on tolerance to high or low temperature conditions that promote the development of these diseases in the field, rather than the genetic capacity to prevent the disease from developing, the level of damage to the bacteria is determined by the complex interaction of climate, variety, management and concentration (Diago *et al.,* 2009; Jaimes y Parra, 2017 and Moreno *et al*., 2013).). Because this study was done with controlled variables, the difference in tolerance/susceptibility observed may be a consequence of the lack of influence of the environmental factors mentioned. With respect to the number of roots, variety F2000 with isolate Bg007 was the most affected in all cases, while variety F68 with isolate Bg010 was the least affected. In terms of severity and different concentrations, the most affected variety was F2000 followed by F67 and the least affected variety was F68. No significant differences were found between severity by concentration in the three varieties, only between the target and the concentrations (Fig.1, Fig. 2).

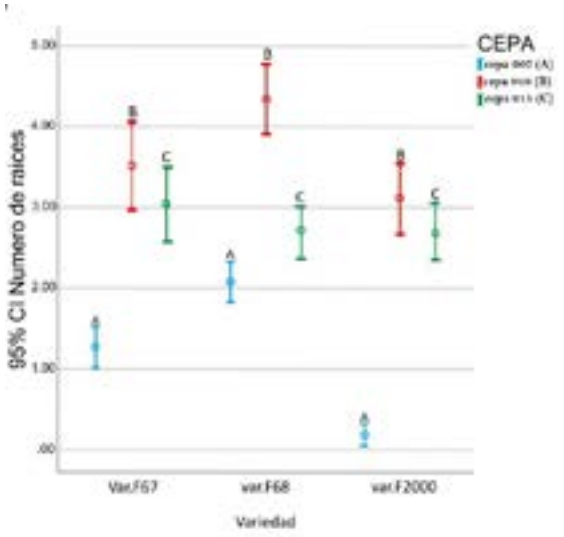

Figure 1. Error Chart for number of roots in the three varieties

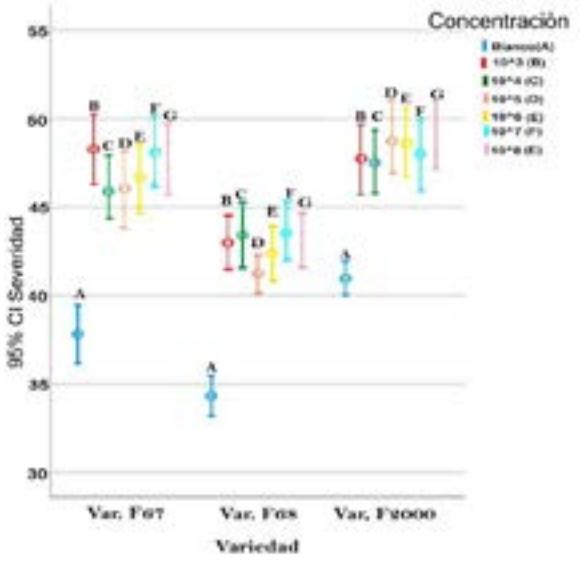

Figure 2. Error Chart for Severity in the different concentrations in the three varieties.

As can be seen the Bg007 (Tab. 1) isolate induced the highest Level 6 counts, especially with the F2000 variety; the Bg010 isolate caused the lowest Level 6 counts, especially with the F68 variety; in the case of Bg011, the highest Level 3 counts were observed, especially with the F68 variety; it is observed that the 010 strain did not cause any Level 3 counts. There were high counts in Level 4 corresponding to severe damage. As it is observed in fig. 2, the seeds had an infection percentage much higher from 5% to 15%, comparing with other works, the isolates used had higher infection capacity, considering that the lower severity obtained in this work was Level 3, which shows that the strain of *Burkholderia glumae* used (Castilla *et al.,* 2010) can be less virulent than those used in this work or that the varieties used in the mentioned work are more resistant to the virulence of *Burkholderia glumae*. In none of the cases observed in the present work showed the 6 levels of severity observed (Kim *et al.,* 2004), all seeds evaluated had virulence greater than or equal to Level 3 showing affectation in all cases. In most cases, inoculum concentration did not significantly influence *Burkholderia*

glumae affectation to rice plant measurements, which significantly affects plant measurements is the strain type of *Burkholderia glumae* and the variety itself.

Table 1. Severity Count

			No Severity	*Mild*	*Moderate*	*Severe*	*Very Severe*	*no Growth*	*Total*
Isolated Bg007	variety	F67	0	0	1	110	19	69	199
		F68	0	0	15	149	6	30	200
		F2000	0	0	0	25	52	114	191
Isolated Bg010	variety	F67	0	0	0	128	24	50	202
		F68	0	0	0	183	0	26	209
		F2000	0	0	0	146	24	35	205
Isolated Bg011	variety	F67	0	0	23	101	17	55	196
		F68	0	0	27	146	19	15	207
		F2000	0	0	0	156	9	42	207

The results obtained agree with what has been reported by several authors who indicate that more than the concentration of inoculum, bacterial grain rotting in rice caused by *B. glumae* is highly dependent on the ability of the bacteria to produce toxin (Mendez-Molina *et al.,* 2020). This is important bearing in mind that it could be a contribution to the Federación Nacional de productores de arroz, which provides advice, training and certified seeds for rice production (Diago *et al.,* 2009). Due to the fact that in the agricultural soils and foliar material from which the isolates evaluated in the present work were obtained, Bg007, Bg010 and Bg011 (Kim *et al.,* 2004) use the F2000 variety for sowing and harvesting, which presented the greatest sensitivity and affectation, it is suggested to use another variety of rice and begin to develop greater prevention to mitigate the possible damage by *Burkholderia glumae*

In order to easily group the measurements and counts obtained in future work, all strains, varieties and dilutions were taken to achieve a measure applicable to susceptible and non-susceptible rice seeds and thus be able to elaborate a quantitative scale of severity. As can be seen in Tab. 2, the measurements were divided into 5 equal parts, i.e. quintiles, which gives a brief description of each severity level.

Table 2. Quintile Frequencies

A*		B*		C*		D*		E		F	
Valid	n	Valid	n	Valid	n	Valid	n	Valid	n	Valid	n
0.00 – 0.99	432	0.00 – 0.99	435	0.00 – 0.99	634	0.00 – 0.99	431	0.00 – 0.99	634	0.00 – 0.99	433
1.00 - 32.77	476	1.00 - 3.75	1136	1.00 - 42.50	512	1.00 - 107.00	926	1.00 - 5.75	878	1.00 - 3.25	1358
32.78 - 65.54	728	3.76 - 7.50	33	42.51 - 85.00	495	108.00 - 214.00	453	5.76 - 11.50	293	3.26 - 6.50	17
65.55 - 98.31	176	7.51 - 11.25	204	86.00 - 127.50	168	215.00 - 321.00	5	11.51 - 17.25	10	6.51 - 9.75	6
98.32 - 131.08	4	11.26 - 15.00	8	127.51 -170.00	7	322.00 - 428.00	1	17.26 - 23.00	1	9.76 - 13.00	2

A. Length of Stem grouped). B. Stem diameter (grouped). C. Length of Main Root (grouped). D. Total Plant Length (grouped). E. Number of Roots (grouped). F. Number of Leaves (grouped). * in millimeters (mm)

Groups the quintiles of each of the variables and a description of each degree of severity (Tab. 3) shows the six variables evaluated in the study (Stem Length, Stem Diameter, Main Root Length, Total Plant Length, Number of Roots and Number of Leaves) and the values for each severity level are presented; five severity levels are expressed with their qualitative description and the value ranges for each variable. From these values a total severity value can be obtained.

Table 3. Quintiles of each variable with its ranges, degrees of severity and description

Degree of Severity	*Qualitative Description*	*Quintiles Length Stem (mm)*	*Quintiles Diameter Stem (mm)*	*Quintiles Length Main Root (mm)*	*Quintiles Total Plant Length (mm)*	*Quintiles Number of Roots*	*Quintiles Number of Leaves*
1	Very considerable Medium growth, very low severity or no severity.	98.32 - 131.08	11.26 - 15.00	127.51 - 170.00	322.00 - 428.00	17.26 - 23.00	9.76 - 13.00
2	Substantial Medium growth, low severity.	65.55 - 98.31	7.51 - 11.25	86.00 - 127.50	215.00 - 321.00	11.51 - 17.25	6.51 - 9.75

3	Average growth, average severity.	32.78 - 65.54	3.76 - 7.50	42.51 - 85.00	108.00 - 214.00	5.76 - 11.50	3.26 - 6.50
4	Low growth, high severity.	1.00 - 32.77	1.00 - 3.75	1.00 - 42.50	1.00 - 107.00	1.00 - 5.75	1.00 - 3.25
5	Very little growth, very high severity or total.	0.00 – 0.99	0.00 – 0.99	0.00 – 0.99	0.00 – 0.99	0.00 – 0.99	0.00 – 0.99

CONCLUSIONS

It could be observed in general that the higher the concentration of *Burkholderia glumae* isolates, the more virulent for the three varieties was Bg007 followed by Bg011 and finally Bg010 with respect to the variety of rice seeds, the most susceptible to the strains was F2000 followed by F67, the most resistant in the present study being F68. Differences were established in the levels of severity of seed infection with respect to the seed variety, isolate used and concentrations evaluated, in the measures found there was no significant difference between dilutions but between strains and varieties. In the measures found there was a significant difference between dilutions and also between strains and varieties. A possible quantitative scale of severity was proposed from the data obtained in the study grouped in quintiles, showing their frequencies and a description of each severity level.

CONFLICT OF INTERESTS:

The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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