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Rise in the Physiological Quality of Seeds of Maize (*Zea mays* L.) Using Agricultural Bioproducts

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Abstract

Context: One of causes of low yields in maize is the poor physiological quality of the seeds, which does not guarantee populations with the required technical parameters. Nationally, seed germination is fostered with the use of bioproducts in different species.
Aim: To determine the vigor and electric conductivity of maize seeds, using bioproducts after putting the seeds under accelerated aging conditions.
Methods: The experiment was done in laboratory III (Bioproducts for Agricultural Use), Faculty of Agricultural Sciences, Jose Marti Perez University of Sancti Spiritus, between October and December 2018. Seeds from four maize varieties provided by the Provincial Seed Company in Sancti Spiritus, were used. Francisco 28 (Fr-28), FgH, MAIG, and P-7928, and were combined with three bioproducts (T. harzianum, FitoMas E, and distilled water). A completely randomized experimental design was used, with factorial arrangement of 4x3x5.
Results: Bioproducts FitoMas E and T. harzianum proved to be effective in the recovery of seeds with physiological deterioration.
Conclusions: T. harzianum offered the best conditions to recover seed vigor, with 20 and 24% in relation to FitoMas E and the distilled water, respectively. The electric conductivity test proved its efficiency as a parameter to determine the vigor of seeds.

Key words: maize, germination, seed aging, Zea mays, Trychoderma.

Introduction

Maize (*Zea mays* L.) belongs to the gramineae family, maideas tribe. It is believed to have originated in the tropics of Latin America, particularly genera Zea, Tripsacum, and Euchlaena, whose importance lies in its phytogenetic relation with genus Zea.

There is experimental evidence that *Trichoderma* spp. stimulates plant germination and growth, along with antagonist activity against pathogen Fusarium spp. (Cubillos, Páez & Mejía, 2011).

Cuba produces and promotes the use of FitoMas-E®, a product containing mineral salts and highly energetic biochemical substances (amino acids, nitrogenized bases, saccharides, and biological active polysaccharides), which increase and speed up seed germination, whether they are botanical or agamic (Viñals Verde et al., 2011).

Seed vigor tests have become common practice tools to determine the physiological quality of seed batches. (Manfrini, 2004).

The electric conductivity test (EC) is suggested to provide germination and/or vigor of seeds in 24 hours or less, and it allows estimation of cell membrane integrity. Its loss and subsequent disappearance of cytoplasmic dissolutes with electrolytic properties indicate quick deterioration of seeds.

In the province of Sancti Spiritus, 29 355 hectares are cultivated with maize, with mean yields of 29 t ha^{-1} in the private sector, and 1.6 t ha^{-1} in the state sector. The mean national values are 2.37 t ha^{-1}, far from the world mean of 5.6 t ha^{-1} (ONE, 2017). One of causes of low yields in maize is the poor physiological quality of the seeds, which does not guarantee populations with the required technical parameters.

Therefore, the aim of this paper is to evaluate the physiological quality of maize seeds from the Seed Company of Sancti Spiritus, using *Trichoderma harzianum* and FitoMas E.

Materials and Methods

The experiment was done in laboratory III (Bioproducts for Agricultural Use), Faculty of Agricultural Sciences, Jose Marti Perez University of Sancti Spiritus, between October and December 2018. Botanical seeds from four maize varieties provided by the Provincial Seed Company in Sancti Spiritus, were used. Francisco 28 (Fr-28), FgH, MAIG, and P-7928.

A completely randomized experimental design was used, with six treatments and five replicas. Germination tests were made on sterile Petri dish with filter paper. It was run at 28 ºC ± 1 ºC in a germination chamber, with light photoperiod of hours/light and 16 hours/dark.

The treatments are the combination of the variety factor with four levels (Francisco-28, FgH, MAIG, and P-7928), and Bioproducts with 3 levels (distilled water, FitoMas E, and *T, harzianum*)

The seeds were previously disinfected with a commercial chlorine solution (5.25% sodium hypochlorite) at 10% for three minutes, and then it was washed three times with sterile distilled water.

Artificial deterioration was induced by accelerated aging. The seeds were chosen according to size uniformity and physical appearance; they were placed on a metallic mesh forming a layer inside a dryer containing 1L of water. The separation of the water from the seed layer was 2 cm to prevent contact with the water, creating an aging chamber with a relative humidity of 100%.

The dryer was introduced in an incubator at 45 ± 1 °C for 72 hours. Upon completing aging, the seeds were exposed to the environment of the lab until recovering their initial contents of humidity (15%).

The doses and application time were,

T. harzianum --- 1.9 x 10^9 conidia per milliliter, the seeds were dipped for 1 hour.

FitoMas E----------solution at 2%, the seeds were dipped for 1 hour.

The following vigor tests were determined as:

Germination Power (GP). It was performed seven days after placing the seed on the dishes, according to Engels & Vissier (2007).

$$P = \frac{N}{NP} * 100$$

Where:

N: number of seeds germinated at seven days

NP: total number of seeds

Mean germination Time (MGT) It is calculated by determining the number of germinated seeds every day, considering the total number of germinated seeds (Tompsett & Pritchard, 1998)

$$TMG = \sum ni.di/N$$

Where:

ni: number of seeds germinated on d day

di: number of days since the beginning of the germination experiment

N: total number of seeds germinated at the end of the experiment

Radicle and coleoptile lengths (cm) The two tests were made on the seventh day following germination, using a ruler. The number of secondary roots were considered for the radicle.

Electric conductivity. A completely randomized experimental design was used, with six treatments and four replicas. Overall 100 seeds were used in each treatment, 25 per replica. After the rest time (up to 15% humidity), 25 seeds were submerged in 50 ml of distilled and deionized water for 24 hours. Then the seeds were removed and their values were read with a portable conductimeter.

A two-way ANOVA was performed for statistical analyses of germination power, mean germination time, and radicle and coleoptile lengths. To check electric conductivity, a one-way ANOVA was made after ensuring homogeneity and normality value checks, through the Levene and Kolmogórov Smirnov test, based on SPSS, 21, for Windows. The mean values were compared through Tukey multiple range test (p≤0.05). The germination percentages mean and mean germination time were transformed by $2\, arcsen\sqrt{p}/100$ to adjust to the normal likelihood curve.

Results and discussion

The statistical analysis of germination power showed interaction among the bioproducts with the varieties used, among the different bioproducts, and among the four varieties of maize. The highest germination potency value was achieved with FgH, using *T. harzianum*, with 82% (Table 1). It was 44% higher than the Fr-28 treatment, with distilled water, the lowest, and no different from FitoMas E, in the same variety (42%). Likewise, MAIG and P-7918 on distilled water showed no differences from the previously mentioned treatments.

Table 1. Germination power.

Varieties	Fr-28	FgH	MAIG	P-7928		
	Germination potency (%)					
Bioproducts					Bioproduct mean	Typical error
Distilled water	38.0C	62.0B	40.0C	42.0C	45.5b	
T.harziarum	62.0B	82.0A	40.0C	70.0AB	65.0a	3.27
FitoMas E	42.0C	72.0AB	58.0BC	70.0AB	60.0c	
Mean of varieties	47.3c	72.0a	48.0c	60.6b	57.0	
Typical error		3.78				
VC (%)						29.0

Unequal capital letters for the means of the interactions differ ($p \leq 0.05$), according to Tukey's multiple range test.
Unequal lower case letters in the row for the means of the varieties differ ($p \leq 0.05$), according to Tukey's multiple range test.
Unequal lower case letters in the row for the means of the bioproducts differ ($p \leq 0.05$), according to Tukey's multiple range test.

Table 1 shows that the bioproduct that reached the best germination power was *T. harzianum* different than Fitomas E and distilled water, respectively. In that sense, trichoderm raised the germination power in FR-28 in 20 and 24%, with respect to FitoMas E and water, respectively, the treatments with the lowest potency.

The previous results differ from González et al. (2014), on evaluation of accelerated aging in maize varieties in Mexico, where the percentages of germination power were greater than 70%. It demonstrates that the seeds evaluated in the Seed Company experiment in Sancti Spiritus were physiologically harmed. During the experimental cycle, the incidence of contaminating fungi was remarkable, which were thought to have come from the seeds, as was suggested by disinfection measures.

The best variety was FgH, which differed from the rest. Aristizábal & Álvarez (2006) said that the seeds with a germination power higher than or equal to 80% after accelerated aging, might be classified as of high vigor; between 60-80% as mid vigor; and 60% as low vigor. In that sense, only FgH on *T. harzianum*, came close.

Concerning mean germination time, interaction was observed between bioproducts and varieties, among varieties, but no interaction was found among the bioproducts (Table 2). In this parameter, there were no statistically different statistics among varieties FgH and P-7928, with none of the bioproducts used. In turn, the treatment with the longest mean germination time was MAIG, with distilled water, more than 1.88-fold FgH treatment with *T. harzianum*, the shortest mean germination time. These results corroborate the criteria of Olmedo and Casas (2014), who said that trichoderm stimulates plant growth and development with the production of molecules that foster plant growth.

In that direction, FgH was the best variety, though no different from P-7928. Moreover, Fr-28 and MAIG reached the highest values with no differences between them. This reveals that the Fr-28 seeds were not physiologically healthy.

Table 2. Mean germination Time.

Varieties	Fr-28	FgH	MAIG	P-7928		
	Mean germination time					
Bioproducts					Bioproduct mean	Typical error
Distilled water	2.62ABC	2.34CDEF	3.35A	1.97EF	2.57a	
T.harzianum	2.87ABC	1.86F	2.66ABC	1.76F	2.29a	0.11
FitoMas E	2.51ABC	2.02CDEF	3.03AB	2.03CDEF	2.39a	
Mean of varieties	2.66b	2.07a	3.01b	1.92a	2.41	
Typical error		0.19				
VC (%)						25.0

Unequal capital letters for the means of the interactions differ ($p \leq 0.05$), according to Tukey's multiple range test.
Unequal lower case letters in the row for the means of the varieties differ ($p \leq 0.05$), according to Tukey's multiple range test.
Unequal lower case letters in the row for the means of the bioproducts differ ($p \leq 0.05$), according to Tukey's multiple range test.

Artola (2015), in a study of different biopreparations of efficient microorganisms, achieved mean germination times of 2.19. Although it was higher than the treatments of varieties FgH and P-2978 with *T. harzianum and* FitoMas E, respectively, the outcome of that research was better than Fr-28 and MAIG, using the same bioproducts. All the varieties used in this study based on distilled water produced higher values, which demonstrates the effectiveness of bioproducts to favor germination in low physiological quality seeds.

The length of the radicle showed interaction among the bioproducts with the varieties used, among the different bioproducts, and among the four varieties of maize (Table 3). The worst variety and differences with the rest, was Fr-28, evidencing, as in the previous parameter, that it was the variety with the lowest physiological quality. For its part, Fitomas E produced the best results, different from the other bioproducts, where distilled water was the shortest in the radicle of all the varieties used, which demonstrates the effectiveness of Fitomas E and *T. harzianum* as growth enhancers.

Table 3. Radicle length.

Varieties	Fr-28	FgH	MAIG	P-7928		
	Radicle length (cm)					
Bioproduct					Bioproduct mean	Typical error
Distilled water	4.10E	7.98BCD	7.68CD	8.10BCD	6.96b	
T.harzianum	6.74D	9.0ABC	7.50CD	9.96AB	7.96b	0.32
FitoMas E	8.16BCD	10.78A	9.96AB	10.68A	9.89a	
Mean of varieties	6.33b	9.25a	8.38a	9.12a	8.27	
Typical error		0.37				
VC (%)						26.3

Unequal capital letter for the means of the interactions differ (p ≤0.05), according to Tukey's multiple range test.
Unequal lower case letters in the row for the means of the varieties differ (p ≤0.05), according to Tukey's multiple range test.
Unequal lower case letters in the row for the means of the bioproducts differ (p ≤0.05), according to Tukey's multiple range test.

Similar stimulation results in radicle length were achieved by Santana et al. (2016) in a study of the same bioproducts, but in tomato; they explained the benefits over different morphological variables evaluated.

As in the previous aspect, coleoptile length showed interaction among the bioproducts with the varieties used, among the four varieties of maize, but not among the bioproducts used (Table 4).

Table 4 Coleoptile length.

Varieties	Fr-28	FgH	MAIG	P-7928		
	Coleoptile length (cm)					
Bioproduct					Bioproduct mean	Typical error
Distilled water	4.5.40B	9.49AB	7.80B	8.0AB	7.49ab	
T.harzianum	5.70B	9.836AB	6.40B	7.20B	7.29ab	0.48
FitoMas E	6.40B	12.10A	8.90AB	7.80AB	8.80a	
Mean of varieties	5.83c	10.48a	7.46b	7.66b	7.86	
Typical error			0.56			
VC (%)						34.0

Unequal capital letter for the means of the interactions differ (p ≤0.05), according to Tukey's multiple range test.

Unequal lower case letters in the row for the means of the varieties differ (p ≤0.05), according to Tukey's multiple range test.

Unequal lower case letters in the row for the means of the bioproducts differ (p ≤0.05), according to Tukey's multiple range test.

The longest treatments were the combination of Fitomas E with FgH, MAIG, and P-7928, with no differences among them, and statistical differences with the rest of the treatments evaluated. The best variety, with significant differences from the rest, was FgH; it was 1.79-fold higher than Fr-28, the variety with the lowest values and statistical differences with the rest.

Santana et al. (2016) showed greater growth and development of tomato plantlets with the use of these bioproducts compared to a control. In contrast to this study, the previous authors found better results using a combination of Fitomas E and *T. harzianum*; hence, this may be considered for further research.

Figure 1 shows the electric conductivity found from seed exudates after undergoing an artificial aging process. FgH reached the lowest value, with statistically significant differences from the rest of the varieties evaluated, followed by P-7928, also different from the rest.

The varieties with the lowest values in this parameter were precisely the ones with the best germination power, along with the longest radicle and coleoptile, and the shortest germination time. It proves that these seeds had the best physiological conditions to withstand aging and a better response to the bioproducts used.

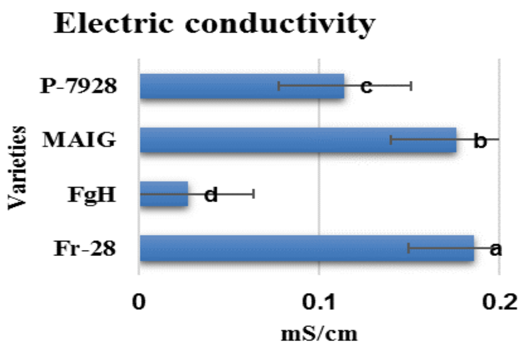

Figure 1. Electric conductivity

The current research coincides with Hilmig & Méndez (2007), who said that the test of electric conductivity allows for the estimation of the integrity of the cell membrane. Its loss and subsequent disappearance of cytoplasmic dissolutes with electrolytic properties indicate quick deterioration of seeds. Therefore, the evaluation of electric conductivity of the seed exudate should be a sign of seed deterioration, and consequently, seed quality.

Conclusions

1. Bioproducts FitoMas E and *T. harzianum* proved to be effective in the recovery of seeds with physiological deterioration.

2. Bioproduct *T. harzianum* offered the best conditions to recover seed vigor, with 20 and 24% in relation to FitoMas E and the distilled water, respectively.

3. The electric conductivity test proved to be an efficient parameter to determine the vigor of seeds.

Author contribution

Michell Leiva Arbolaes: practical execution of the research, literature review or state of the art.

Marcos T. García González: Research planning, direction, and control of research, analysis of results, manuscript redaction, final review.

Marcia M. Rodríguez Jáuregui: Direction, advisory, and control of laboratory research.

Yander Fernández Cancio: Statistical analysis and collaboration in the practical part of research.

Conflicts of interest

There are no conflicts of interest.

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