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Response of seed imbibition time of three citrus rootstocks with different concentrations of Pectimorf® during germination

Respuesta del tiempo de imbibición de semillas de tres patrones de cítricos con diferentes concentraciones de Pectimorf® en la germinación

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Abstract: Finding safer germination methods is essential in citrus fruits, so the aim of this research was to evaluate the response of the imbibition time of seeds from three citrus rootstocks with different concentrations of Pectimorf® on germination. An experiment was conducted in the laboratory with two study factors: Factor A: rootstock seed (A1: Cleopatra mandarin, A2: rough lemon, A3: mandarin lime) and Factor B: pectic oligosaccharide concentration (B1: 0 mgL-1, B2: 5 mgL-1, B3: 10 mgL-1, B4: 20 mgL-1). The treatments were distributed in a completely randomised experimental design (CRD) in a 3x4 factorial arrangement, with 12 treatments. The percentage of germination (PDG), radicle wet weight (PHDR), radicle dry weight (PSDR), vigour index (IDV) and seed growth dynamics were evaluated. The analyses were performed in Infostat and Excel. The results showed that the lime mandarin rootstock performed best at a dose of 20.00 mgL-1. The rough lemon performed best at doses of 5.00, 10.00 and 20.00 mgL-1, and the Cleopatra mandarin at doses of 10.00 and 20.00 mgL-1. With regard to germination speed, rough lemon responded best to 5.00 mgL-1 of polysaccharides, followed by rough lemon with 10.00 mgL-1 of oligosaccharides. The application of the biostimulant Pectimorf® to lime mandarin seeds had the best pHDR weight- .

Keywords: imbibition, seeds, oligosaccharides, germination, citrus fruits

Resumen: Encontrar métodos de germinación más seguras es fundamental en cítricos, por lo que la presente investigación tuvo el objetivo de evaluar la respuesta del tiempo de imbibición de semillas de tres patrones de cítricos con diferentes concentraciones de Pectimorf® en la germinación. Se implementó en laboratorio un experimento, con dos factores de estudio: Factor A: semilla de patrones (A1: mandarina Cleopatra, A2: limón rugoso, A3: lima mandarina) y Factor B: concentración de oligosacáridos pécticos (B1: 0 mgL-1, B2: 5 mgL-1, B3: 10 mgL-1, B4: 20 mgL-1). Los tratamientos se distribuyeron en un diseño experimental

Completamente Aleatorio (DCA) en arreglo factorial 3x4, con 12 tratamientos. Se evaluó el porcentaje de germinación (PDG), peso húmedo de radícula (PHDR), el peso seco de radícula (PSDR), el índice de vigor (IDV) y la dinámica de crecimiento de la semilla. Los análisis se realizaron en infostat y excel. Los resultados mostraron que el patrón lima mandarina fue mejor a la dosis de 20,00 mgL-1. El limón rugoso fue mejor en las dosis 5,00, 10,00 y 20,00 mgL-1 y la mandarina Cleopatra a las dosis de 10,00 y 20,00 mgL-1. Respecto de la velocidad de germinación, hubo mejor respuesta del limón rugoso con 5,00 mgL-1 de polisacáridos, seguidos del limón rugoso con 10,00 mgL-1 de oligosacáridos. La aplicación del bioestimulante Pectimorf® a la semilla de lima mandarina tuvo mejor peso PHDR.

Palabras clave: imbibición, semillas, oligosacáridos, germinación, cítricos

Introduction

Biostimulants are microorganisms or substances that promote plant nutrition, confer tolerance to abiotic stress, and increase crop yield and quality (Jardín, 2015, Yakhin 2017). Microbial biostimulants, such as rhizobium-based inoculants, for example, are frequently used to reduce the use of inorganic fertilisers. These microorganisms have the ability to carry out biological nitrogen fixation (BNF) in symbiosis with leguminous plants and other crops. Through this process, they supply the plant with atmospheric nitrogen in an assimilable form, thus promoting its growth and development (Zaccardelli et al., 2013).

Among the most widely used non-microbial biostimulants in agriculture are oligosaccharides (Mederos and Hormaza, 2008, Falcón Rodríguez et al., 2015). Among these molecules, the most studied for their biological applications are oligogalacturonides, chitins and chitosans, and nodulation factors from the Rhizobiaceae family (Falcon Rodríguez et al., 2015). Oligogalacturonides (OGAs) can regulate hormone synthesis and action and various organogenesis and growth processes in plants (Fundora et al., 2013). The mixture of OGAs, commercially known as Pectimorf®, stimulates rooting, growth and cell differentiation in different plant species (Falcón Rodríguez et al., 2015, Fundora et al., 2013, Falcón Rodríguez et al., 2013, Nápoles Vinent et al., 2016). In addition, it can activate defence mechanisms and reduce or mitigate environmental stress in plants (Falcón Rodríguez et al., 2015, Terry-Alfonso et al., 2014a, Terry-Alfonso et al., 2014b).

According to Jaramillo et al. (2012), within the life cycle of plants, the germination process is very vulnerable, as the development of a new species depends on it. Seed germination depends on influential factors such as the environment, storage time, and the temperature at which they are dried. In this sense, knowledge of the effect of biofertilisers on imbibition, germination, and plant growth, as well as proper management, will be vital for obtaining quality plants with adequate size and root systems, according to Rizo et al. (2018), the most commonly used products for this purpose are Azofert®, EcoMic® and Pectimorf®.

Pectimorf® has been used successfully in different biotechnological processes such as somatic embryogenesis in sugar cane (Saccharum spp.) (Nieves et al., 2006) and mandarin (Citrus reshni Hort.), (Hernández et al., 2007). Furthermore, the addition of a mixture of oligogalacturonides to the culture medium stimulated the number of shoots per explant in the accelerated micropropagation of Anthurium cubense (Montes, 2000). Pectimorf® has been shown to stimulate rooting and promote the subsequent acclimatisation of FHIA-18 banana in vitro plants (Borges et al. 2015) and papaya cv. Maradol Roja shoots (Posada Pérez et al., 2016). Pectimorf® (10 mg L-1) also stimulated rooting of cuttings in the Ficus benjamina Golden King and Nítida varieties (Dominí and Benítez, 2004) and in African violet petioles (Saintpaulia ionantha L.) (Pérez et al., 2021).

However, despite the use of Pectimorf® in several species at different concentrations, application methods and growing conditions, no study has yet been conducted to determine the concentrations that favour seed germination in citrus fruits, with the exception of the work carried out by Alcivar and Vera (2022), who studied the response of seed imbibition of three citrus rootstocks with different concentrations of Pectimorf® on seed germination. They found that rough lemon seeds had a germination rate of 56.56% after 15 days in a glass substrate. In order to confirm and improve germination in different citrus rootstocks, this research was conducted to evaluate the response of the imbibition time of seeds from three citrus rootstocks with different concentrations of Pectimorf® on germination.

Methodology

This research was conducted at the Universidad Estatal Del Sur de Manabí (UNESUM), located 1 ½ km via Noboa, Los Ángeles, Cantón Jipijapa, Province of Manabí. It is located at 1°21'10.14" south latitude

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and 80°33'50.40" west longitude at an altitude of 313 metres above sea level (Gabriel et al., 2024).

The study factors were the patterns (A1: Cleopatra mandarin, A2: rough lemon, A3: lime mandarin) and the concentrations of pectic oligosaccharides (B1: 0 mgL-1, B2: 5 mgL-1, B3: 10 mgL-1, B4: 20 mgL-1).

Treatments

The 12 treatments used are detailed in Table 1.

Table 1. Research treatments .

No.	Treatment	Factor A (seed)	Factor B (dose) (1000 mL)	Factor B (dose) (336 mL)
1	A1 x B1	Cleopatra mandarin (*Citrus reshni*)	0 mg L^{-1}	0mg L^{-1} , $^{-1}$
2	A1 x B2	Cleopatra mandarin (*Citrus reshni*)	5 mg L^{-1}	1.68 mg L^{-1} ,
3	A1 x B3	Cleopatra mandarin (*Citrus reshni*)	10 mg L^{-1}	3.36 mg L^{-1} ,
4	A1 x B4	Cleopatra mandarin (*Citrus reshni*)	20 mg L^{-1}	6.72 mg L
5	A2 x B1	Rough lemon (*Citrus x jambhiri)*	0 mg L^{-1}	0mg L^{-1} , $^{-1}$
6	A2 x B2	Rough lemon (*Citrus x jambhiri*)	5 mg L^{-1}	1.68 mg L^{-1} ,
7	A2 x B3	Rough lemon (*Citrus x jambhiri*)	10 mg L^{-1}	3.36 mg L^{-1} ,
8	A2 x B4	Rough lemon (*Citrus x jambhiri*)	20 mg L^{-1}	6.72 mg L
9	A3 x B1	Lime (*Citrus × limonia*)	0 mg L^{-1}	0mg L^{-1} , $^{-1}$
10	A3 x B2	Lime (*Citrus × limonia*)	5 mg L^{-1}	1.68 mg L^{-1} ,
11	A3 x B3	Lime (*Citrus × limonia*)	10 mg L^{-}	3.36 mg L^{-1} ,
12	A3 x B4	Lime (*Citrus × limonia*)	20 mg L^{-1}	6.72 mg L

Research management

The Mandarina Cleopatra, Rough Lemon, and Mandarin Lime citrus rootstocks were disinfected using a 25% sodium hypochlorite solution for 2 minutes, then washed with distilled water and left to dry at room temperature. The disinfected seeds were soaked for the time established based on previous results using different concentrations of Pectimorf® ( ) (0, 5, 10, 20 mgL-1) (Alcivar and Vera, 2022). Once the soaking was complete, the seeds were placed in Petri dishes (100 mm in diameter) with filter paper and distilled water was added. Twenty seeds were used per dish and four dishes per treatment, making a total of 320 seeds per pattern. The dishes were kept at a temperature of 26°C to promote seed germination.

Experimental design

The experiment was implemented in a completely randomised design (CRD) in a 3x4 factorial arrangement with 12 treatments and four replicates (Gabriel et al., 2022).

The response variables evaluated were as follows: Germination percentage (%) (PDG). To determine this variable, the number of germinated seeds was counted and related to the total number of seeds treated, then multiplied by 100 to convert to a percentage (Yanez and Vera, 2023). Wet weight of radicle (g) (PHDR). The wet weight of each radicle was determined for all treatments using an analytical balance (Yanez and Vera, 2023). Dry weight of radicle (g) (PSDR). The radicles were dried in a muffle furnace at 70 °C. They were subjected to this temperature until they reached a constant weight. They were then weighed using an analytical balance (Yanez and Vera, 2023). Vigor index (IDV). This value was obtained by relating the germination percentage and the dry mass of the radicle (Yanez and Vera, 2023). Seed germination dynamics. This was determined by relating the days to germination and the germination percentage of each of the treatments, determining the best fit curve model.

Statistical analysis

Based on the defined model, analyses of variance (ANOVA) were performed to test hypotheses about fixed effects, as well as comparisons of treatment means using Tukey's test ($P<0.05$). The ANOVA was also used to estimate the variance components for random effects. The analyses were performed using Infostat software (Gabriel et al., 2022).

Normality and homogeneity of variance analyses

The normality (Shapiro-Wilk, $P<0.05$) and homogeneity of variances (Levene, $P<0.05$) analyses did not show significant differences in the

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data for the variables evaluated. This suggested the continuation of the ANOVA and the comparison of means using Tukey's multiple test (P<0.05).

Results

Table 2 shows that the ANOVA revealed highly significant differences (P<0.01) for Factor A (Patterns) in the PDG, PHDR and PSDR variables. The IDV was not significant.

In the case of Factor B (oligosaccharide concentration), there were highly significant differences (P<0.01) for PDG (Table 2). No significant differences (P<0.05) were detected for the interaction of Factors A x B. The coefficients of variation (CV) ranged from 6 to 27%, which is adequate for this type of research (6 to 27%).

Table 2. ANOVA of PDG, PHDR, PSDR, and IDV.

FV	**gl**	**Mean squares**			
		PDG	**PHDR**	**PSDR**	**IDV**
Model	11	23.29**	3.0E-05*	1.8E-06ns	0.16ns
A	2	103.15**	1.1E-04**	5.5E-06**	0.44ns
B	3	13.00**	1.5E-05ns	1.7E-06ns	0.14ns
A x B	6	1.81ns	1.0E-05ns	7.1E-07ns	0.08ns
Error	36	1.15	1.3E-05ns	8.0E-07ns	0.10ns
Total	47				
CV		6.47	27.09	26.36	14.67

**: highly significant (P<0.01),*: significant (P<0.05), ns: not significant, GPR: germination percentage, RHW: radicle wet weight, DRW: radicle dry weight, VRI: vigour index.

Table 3 shows the comparison of means using Tukey's multiple range test (P<0.05), where it can be seen that the Lima Mandarina rootstock was outstanding for PDG, PHDR, PSDR and IDV.

Table 3. Analysis of means for evaluated rootstocks.

Rootstocks	PDG	PHDR	PSDR	IDV
Lima Mandarina	**95.00 to**	**0.02 to**	**3.9E-03 to**	**2.31 to**
Rough Lemon	84.05 ab	0.01 b	3.5E-03 ab	2.06 ab
Cleopatra mandarin	69.70 c	0.01 b	2.8E-03 b	2.00 b
DSH	0.93	0.0031	0.00077	0.27

Means with a common letter are not significantly different (P < 0.05), PDG: germination percentage, PHDR: radicle wet weight, PSDR: radicle dry weight, IDV: vigour index.

Table 4 shows the comparison of means using Tukey's multiple test (P<0.05) for oligosaccharide concentration, which shows that there were significant differences for PDG, where the concentration of 20.00 mgL-1 was the best, achieving up to 95% germination, in reference to the other concentrations evaluated.

For oligosaccharide concentrations in PDHR, PSDR, and IDV, there were no significant differences (P<0.05) (Table 4).

Table 4. Analysis of means for oligosaccharide concentration (mgL^{-1}).

Oligosaccharide concentration (mgL^{-1})	PDG	PHDR	PSDR	IDV
20.00	**89.15 to**	0.01 to	3.5E-03 to	2.25 to
10.00	84.15 ab	0.01 to	2.9E-03 to	2.17 to
5.00	81.65 bc	0.01 to	3.8E-03 to	2.08 to
0.00	56.65 c	0.01 to	3.3E-03 to	2.00 to
DSH	1.1805	0.01	0.61	0.34

Means with a common letter are not significantly different (P <0.05). PDG: germination percentage, PHDR: radicle wet weight, PSDR:

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radicle dry weight, IDV: vigour index, DSH: honest significant difference.

Seed germination dynamics

With regard to seed germination dynamics (Figure 1), six of the treatments were outstanding at 21 days of evaluation. In general, all fit better to a straight line with coefficients of determination close to 1.00 (R2 = 1). It was observed that the Lima mandarin pattern had better germination over time at a dose of 20.00 mgL-1, achieving up to 89.15% germination. The rough lemon showed increasing performance at doses of 5.00, 10.00 and 20.00 mgL-1, and the Cleopatra mandarin at doses of 10.00 and 20.00 mgL-1.

Likewise, the rates of change or slope of the curve indicate the germination rate over time. Thus, in the case of Figure 1, treatment E (A3B2) had the highest germination rate (1.0379x), indicating that the rough lemon treatment with 5.00 mgL-1 of polysaccharides was outstanding, followed by treatment D (A2B3), which corresponds to rough lemon and 10.00 mgL-1 of oligosaccharides.

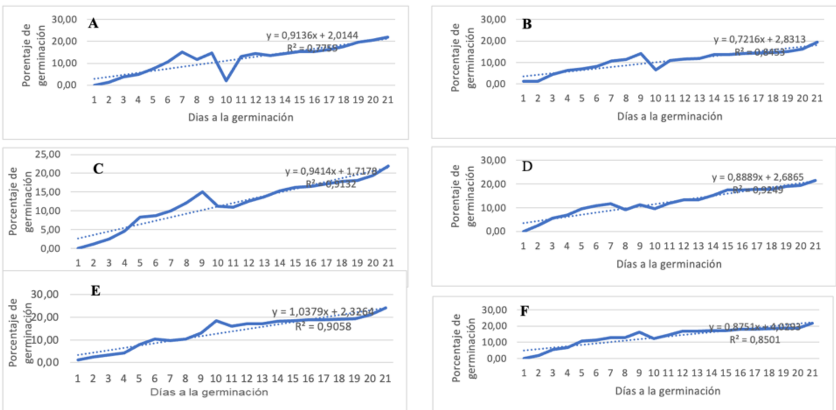

Figura 1. Dinámica de la germinación de la semilla. **Figura 1.** . **A:** A1B3, **B:** A2B2, **C:** A2B3, **D:** A2B4, **E:** A3B2, **F:** A3B3

Discussion

According to the results of the research on germination and weight of citrus rootstocks as a function of imbibition at different doses of Pectimorf®, the mandarin lime seed rootstock obtained the highest average in terms of germination percentage, wet and dry radicle weight, and the highest vigour index.

The research showed that the lime mandarin rootstock had the highest germination percentage of all the rootstocks at 19%, while the Cleopatra mandarin rootstock had the lowest germination percentage. In terms of oligosaccharide concentration, the 20 mg L-1 dose of Pectimorf® had the highest germination percentage and the lowest was the control. According to Alcívar and Vera (2022), the germination percentage of rough lemon ( ) was the most significant of the rootstocks, with 56.56% at a dose of 10 mg L-1 of Pectimorf®. Farías (2019) and Borges et al. (2016) agree with this author, reporting that 80% of rough lemon seeds germinated in less time when using an organic stimulant based on nettle and horsetail extract. Calderón (2018) determined that seeds immersed in lemon seed extract (Citrus limon) for 12 hours achieved 80% germination, acting as a germination accelerator.

In terms of PHDR and PSDR, there were no significant differences between the rough lemon and Cleopatra mandarin rootstocks. Lima mandarin was different, with a higher weight than the other rootstocks. No statistical differences were obtained for both variables in terms of oligosaccharide concentration. Núñez et al. (2018) indicated that the PSDR weight of rice cv. INCA LP-7, stimulated with NaCl 100 mmol L-1 for 13 days, significantly affected radicle length. For Farías (2019), the application of 50% and 100% nettle extract resulted in a significant stimulation of the dry biomass of the aerial part and root of the rough lemon seedling.

For IDV, according to the analysis of variance, there were no statistical differences for the rootstocks, concentration, and interaction of both. However, lime mandarin had the highest percentage at 2.32% compared to the other rootstocks, and the concentration with higher results than the rest was for the 20 mgL-1 dose of Pectimorf®. For Alcívar and Vera (2022), rough lemon, with 4.13% germination vigour, was the best compared to other citrus rootstocks. In terms of the interaction between rootstocks and Pectimorf® doses, there was no statistically significant difference, indicating that the amount of this biostimulant does not influence citrus rootstocks.

Other research highlights the action of the bioactive Pectimorf®. Terry-Alfonso et al. (2014a, 2014b) indicated that it has a positive effect on radish cultivation (Raphanus sativus L.), stimulating the length of the hypocotyl and radicle in seedlings. It also stimulates plant growth and development, as well as agricultural yield and its components, exceeding crop production by 40-50% after application of this bioactive product obtained in Cuba, which demonstrates its effectiveness as a biostimulant. Bao (2013) stated that both Pectimorf® and

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brassinosteroids at the concentrations used accelerate and increa ly the process of somatic embryogenesis in vitro of Citrus macrophylla Wester.

Conclusions

It was determined that the application of the biostimulant Pectimorf® to mandarin lime seeds resulted in better PHDR.

In terms of seed germination dynamics, six of the treatments stood out in the 21-day evaluation, showing that all followed a straight line.

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