



Research Article

Effect of Culture Medium, pH and GA₃ on Germination and Seedling Growth of *Capsicum frutescens*

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Abstract | *Capsicum frutescens* L. has a great relevance in terms of nutritional value and economic importance worldwide. *In vitro* culture is one of the methods used to reproduce high-quality materials. The objective of this study was to evaluate the effect of different pH levels of the Murashige and Skoog (MS) culture medium on the germination and growth of *C. frutescens* seedlings. Six accessions of *C. frutescens* (ECU-2259, ECU-12967A, ECU-12967B, ECU-2237, ECU-12974, and ECU-11994A) from National Institute of Agricultural Research (INIAP) Gene Bank were studied in a tri factorial experiment distributed on a completely random design was performed (genotypes * pH * GA₃). The seeds were disinfected with 70% ethyl alcohol (30 seconds), 1% sodium hypochlorite (NaClO) with two drops of Tween-20 for 15 minutes, and sterile distilled water, and then sown in test tubes containing MS medium with pH 3.5, 4, 5.8, 6.5, and 7.5, with and without GA₃ (0.75 mg/L). Results showed that germination and seedling height are determined by the characteristics of each accession, with accessions ECU-12967A and 12967B having highest germination rate and speed germinations. Accession 12967A and 12967B were not affected by pH different pH levels. In conclusion, *C. frutescens* plant tolerance to different pH levels can help to reduce damages in vegetative growth and yield parameters during production.

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Introduction

The genus *Capsicum* includes commercially important vegetables known as peppers and chili

peppers (Tripodi and Kumar, 2019) with high nutritional, ornamental, and medicinal value (Gris et al., 2021). Five cultivated species (*Capsicum annuum* L., *Capsicum baccatum* L., *Capsicum chinense* Jack, *Cap-*

sicum frutescens L., and *Capsicum pubescens* Ruiz and Pav) and about 30 wild species are recognized (Carriazo *et al.*, 2016). *C. frutescens* of great relevance in terms of nutritional value and economic importance is cultivated worldwide (Olatunji and Afolayan, 2019; García-González and Silvar, 2020). However, under cultivation, this species is exposed to abiotic factors such as drought and high temperatures that directly affect its growth and productivity (Robledo, 2020).

Peppers can be reproduced through asexual and asexual or vegetative propagation (Chauhan *et al.*, 2021). *In vitro* culture is one of the methods used to asexually reproduce high-quality materials (Haque and Ghosh, 2018). The use of appropriate culture media, such as the Murashige and Skoog (1962) formulation, is essential for the success of *in vitro* plant regeneration and propagation (Ramírez *et al.*, 2012). Culture media contain inorganic salts, organic compounds, growth regulators, vitamins, organic supplements, and gelling agents, but pH is one of the most important factors in their preparation (Ramírez *et al.*, 2012). Ebrahim and Ibrahim (2000) mention that *in vitro* growth of species depends on pH, with slightly acidic values being optimal for root formation. Ambrósio and Melo (2004) affirm that pH of the medium can significantly affect the efficacy of multiplication, establishment, growth, and differentiation of *in vitro* cells. On the other hand, germination rate of pepper seeds has been significantly influenced by pH of the solution, varying from 55% at pH 3.0 to 90% at pH 6.5. Seedling growth showed greater sensitivity to pH, as indicated by notable decreases in root and shoot dry mass, root and shoot length, number of leaves per plant, lateral root formation, and root surface area (Ünlü *et al.*, 2010). Bamidele and Eguajie (2016) demonstrated that low or high pH levels affected all growth parameters of the plant, including plant height, number of leaves, and the fresh and dry weight of leaves, roots, stems, and fruits. Leaf area and fruit production were also impacted. Additionally, nutritional analysis of the leaves revealed a decline as acidity levels increased. Acid soil toxicity is not a singular issue but rather a complex interplay of factors that can affect plant growth. Aluminum and hydrogen ion toxicity, low nutrient availability, and inhibited nutrient uptake are generally regarded as the primary growth limitations within the soil acidity complex (Malkanthi *et al.*, 1995).

The low and slow germination of seeds and emergence

of *C. frutescens* seedlings represent a major obstacle for its domestication and commercial production (Robledo, 2020; Corozo-Quiñónez *et al.*, 2023). Therefore, it is essential to study propagation alternatives such as *in vitro* culture of plant tissues (Calva *et al.*, 2005; Argüelles *et al.*, 2020). The objective of this research was to evaluate the effect of different pH levels of the Murashige and Skoog (MS) medium and GA₃ on the germination and growth of *C. frutescens* L. seedlings.

Materials and Methods

Plant material

The experiment was conducted in Biotechnology Laboratory of the Faculty of Agronomic Engineering (FIAG) at the Technical University of Manabí-Ecuador. Six accessions of *Capsicum frutescens* (ECU-2259, ECU-12967A, ECU-12967B, ECU-2237, ECU-12974, and ECU-11994A) from the Gene Bank of National Institute of Agricultural Research (INIAP) were used. The seeds were washed with water and liquid soap for 2 minutes, rinsed with running water, and then in a laminar flow hood, they were immersed in 70% ethanol for one minute, rinsed with sterile distilled water, and placed in a 1% sodium hypochlorite (NaOCl) solution with two drops of Tween-20 for 15 minutes, washing them three times with sterile distilled water.

Preparation of the culture medium

To prepare Murashige and Skoog (MS) culture medium, macro and micronutrient salts, vitamins, and sucrose were mixed in a volume of 800 mL of distilled water. Subsequently, gibberellic acid (GA₃) was added and the volume was adjusted to 1000 mL. The pH of the culture medium was adjusted to the values of the treatments to be evaluated (3.0, 4.0, 5.8, 6.5, and 7.5) with 1 N hydrochloric acid (HCl) and sodium hydroxide (NaOH). The culture medium was sterilized in an autoclave at 121°C for 20 minutes.

Experimental design

The experiment was conducted in a completely randomized design. The experiment was composed of three factors: *C. frutescens* accessions, pH levels and addition of GA₃, which are detailed below in Table 1.

Sowing and culture conditions

The disinfected seeds were sown under laminar flow hood conditions. A total of 30 seeds from each accession were sown in 150 x 25 mm test tubes containing

10 mL of culture medium. After sowing, tubes were sealed with Parafilm and then incubated under culture conditions at a temperature of $24 \pm 2^\circ\text{C}$, a wrapped of 16 hours light, and a light intensity of 2000 LUX.

Table 1: Experimental factors evaluated in *C. frutescens* under varying pH levels and GA_3 concentrations.

Study factors		
<i>C. frutescens</i> cultivars	pH levels	GA_3
ECU-2259	3.0	0.75 mg L ⁻¹
ECU-12967A	4.0	0 mg L ⁻¹
ECU-12967B	5.8	
ECU-2237	6.5	
ECU-12974	7.5	
ECU-11994A		

Variable's assessment

Microbial contamination, phenolic oxidation, plant height (cm), and new leaf production were evaluated. To evaluate germination, the following equations proposed by [Lozano-Isla et al. \(2018\)](#) were used:

Germination (%G)

$$\text{Germination (\%)} = \frac{\sum_{i=1}^k n_i}{N} \times 100$$

Mean germination time (MGT)

$$\text{MGT} = \frac{\sum_{i=1}^k n_i t_i}{\sum_{i=1}^k n_i}$$

Mean germination rate (MGR). Is expressed as the reciprocal of MGT

Germination speed index (GSP)

$$\text{GSP} = \frac{\sum_{i=1}^k G_i}{\sum_{i=1}^k G_i X_i}$$

Where:

n_i = Number of seeds germinated in the i^{th} time; N = Total number of seeds in each experimental unit; k = Last day of germination evaluation; t_i =Time elapsed from the start of the experiment to the i^{th} observation; f_i = Relative frequency of germination; G_i =Number of seeds germinated in the i^{th} time; X_i = Number of days from sowing.

Statistical analysis

Data were subjected to normality tests (Shapiro-Wilk test) and homogeneity of variances (Bartlett test) before the analysis of variance (ANOVA). When these assumptions were not met, the data were transformed using $\sqrt{y + 0.5}$. When the ANOVA was significant, Scott-Knott's multiple comparison test ($p \leq 0.05$) was applied. Statistical analyses were performed using the "ExpDes.pt" statistical package available in R 4.3.2, and graphs were created in Graphpad Prism (version 8.0.2, San Diego, CA, USA).

Results and Discussion

Seed disinfestation

Achieving a low contamination rate (3%) and a complete absence of phenolic oxidation (0%) in all evaluated accessions of *C. frutescens* constitute a significant result in the *in vitro* establishment of *Capsicum* accessions. These results highlight the effectiveness of the disinfestation protocol employed, which consisted of a precise sequence of treatments with 70% ethanol and 1% sodium hypochlorite (NaClO).

Germination

The variables associated with the germination process of *Capsicum* seeds, such as germination percentage, mean germination time (MGT), mean germination rate (MGR), and germination speed (GSP), were significantly affected ($p \leq 0.05$) by gibberellic acid (GA_3), genotype, and pH level. The GA_3 *genotype interaction showed statistical significance ($p < 0.01$) for germination percentage, MGT, MGR, and GSP, while the genotype * pH level interaction also presented significant differences ($p < 0.01$) for the variables GER and MGT. Finally, the GA_3 *genotype*pH level interaction only affected the germination percentage variable ([Table 2](#)).

The germination percentage was significantly affected by the GA_3 *genotype*pH level interaction ($p < 0.001$). *Capsicum* seeds showed a higher germination percentage in GA_3 - treatments, except for the ECU-2237 genotype. Genotypes ECU-12967A and ECU-12967B did not show differences at pH levels 3.5, 4.0, 5.8, 6.5, and 7.5, with mean germination rates of 87.5, 95.0, 90.0, 75.0, and 80.0%, respectively. ECU-11994A genotype showed the highest germination at pH 4.0 with 55%. The remaining pH levels presented an average germination of 29.0%. The treatments with the lowest germination rates were genotypes ECU-2259,

Table 2: Probability values (p -values) from the analysis of variance (ANOVA) for the effect of pH on indices germination of six *Capsicum* genotypes with and without growth regulator (GA_3) under in vitro conditions.

Source of variation	Indices			
	Germination (%)	Mean germination time (days)	Mean germination rate (days ⁻¹)	Germination speed (%)
Gibberellic acid (GA_3)	0.2708	0.0106**	0.5644	0.6564
Genotype	0.0000***	0.0000***	0.0000***	0.0000***
pH level	0.0004***	0.0334*	0.0023**	0.0000***
GA_3 *genotype	0.0000***	0.0050**	0.0000***	0.0000***
GA_3 *pH level	0.7975	0.0777	0.7898	0.9030
Genotype*pH level	0.0015**	0.0076**	0.3378	0.3013
GA_3 *genotype*pH level	0.0000***	0.5691	0.2041	0.1685
C.V. (%) ⁺	35.65	69.77	54.76	47.05

* $p \leq 0.05$; ** $p \leq 0.01$; $p \leq 0.001$ ***; + Coefficient of variation.

ECU-2237, and ECU-12974, with an average of 12.33% considering all pH levels (Figure 1A). In GA_3 -free treatments, genotypes ECU-12967A and ECU-12967B did not show significant variations in germination with different pH levels, with an average of 85.5%. Genotype ECU-2237 genotype showed the highest germination percentage at pH 4.0 and 5.8 with 32.5%. Meanwhile, genotypes ECU-2259, ECU-11994A, and ECU-12974 showed an mean of 7.66% germination (Figure 1B).

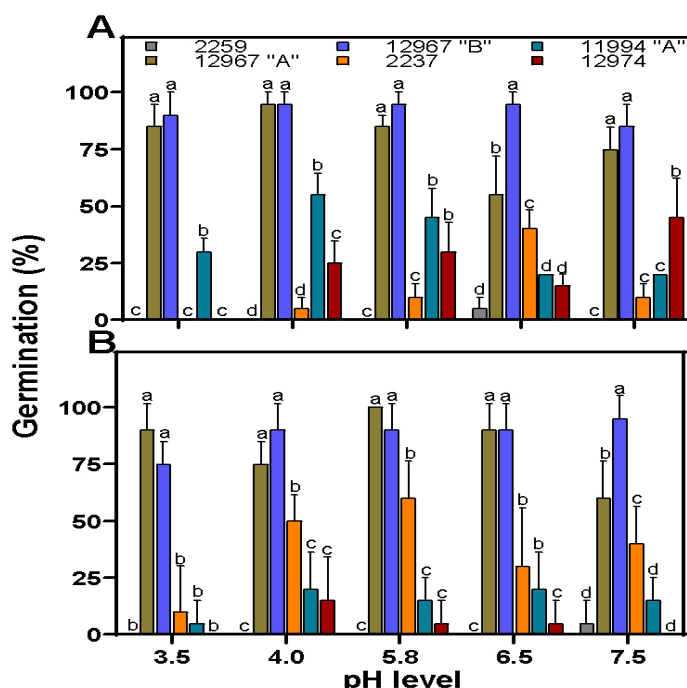


Figure 1: Effect of pH levels on the germination (%) of *Capsicum* spp. genotype seeds treated with (A) and without (B) addition of gibberellic acid (GA_3 ; 0.75 mg L⁻¹) under in vitro conditions. Identical letters within the same pH level do not differ statistically according to the Scott-Knott test ($p \leq 0.05$).

Seed germination of *Capsicum* genotypes was accelerated by the growth regulator (GA_3). In hormone-treated seeds, the germination process started 4 days after sowing, especially in seeds sown at pH 4.0. At pH levels 4.0 and 5.8, average germination rates of 45.83 and 41.16%, respectively, were reached (Figure 2A). The lowest germination percentage was observed at pH 3.5 with 34.16%. In hormone-free seeds, germination began 5 days after sowing. Under these conditions, pH 5.8 showed a final germination percentage of 45%. The intermediate germination percentage was observed at pH 6.5, with an average of 39.16%. Meanwhile, the lowest germination percentage was shown at pH 3.5 with 30% (Figure 2B).

Mean germination time (MGT) was significantly affected ($p < 0.01$) by the genotype* GA_3 interaction. In general, the highest MGT was observed in all genotypes using GA_3 , except for ECU-2237. The genotype with the highest MGT was ECU-11994A with 14.32 days, higher than the treatment without GA_3 application (8.50 days). Meanwhile, genotypes ECU-12967A, ECU-12967B, ECU-2237, and ECU-12967 presented an average MGT of 9.87, with MGT higher than their respective treatments without GA_3 , except the ECU-2237, where the treatment without GA_3 was higher than its counterpart with GA_3 . Conversely, the lowest MGT was observed for ECU-2259, with a mean of 0.40 and 1.00 MGT for treatments with and without GA_3 , respectively (Figure 3A). At pH 3.5, genotypes ECU-12967A, ECU-12967B, and ECU-11994A presented an average MGT of 11.92, 12.26, and 10.05 days, higher than the other treatments. At pH 4.0, the genotypes presented an average MGT of 10.26, higher than the

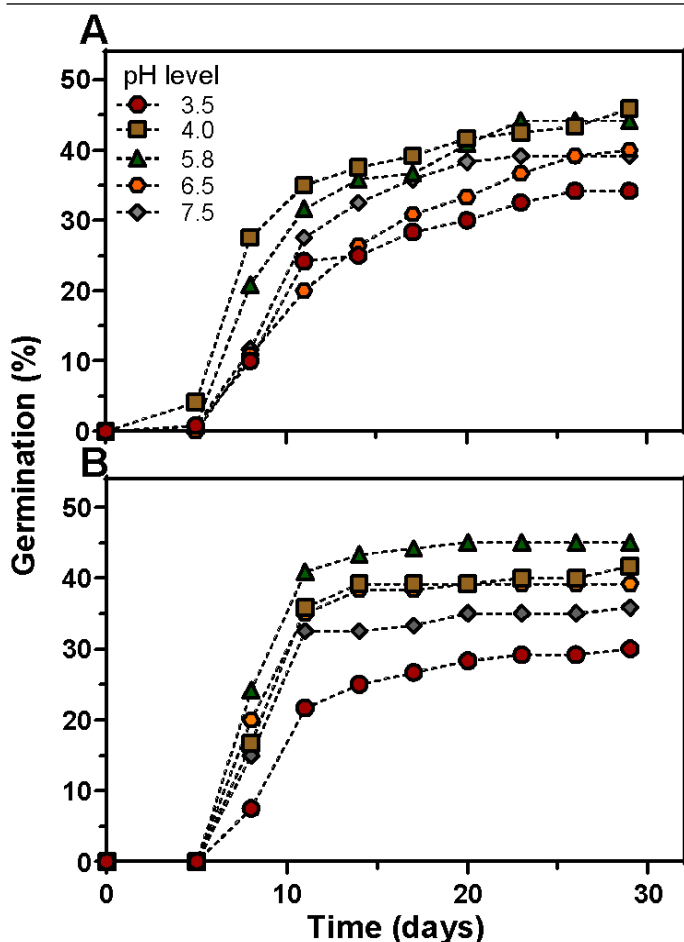


Figure 2: Germination curves of *Capsicum* spp. seeds at different pH levels with (A) and without (B) the addition of gibberellic acid (0.75 mg L^{-1}) under in vitro conditions.

ECU-2259 genotype with an MGT of 0. Similar results were observed at pH levels 5.8, 6.5, and 7.5, where all treatments presented an average MGT of 10.48, higher than the ECU-2259 genotype with average MGT < 2.50 (Figure 3B).

Mean germination rate (MGR) showed significant differences due to the genotype*GA₃ interaction ($p < 0.001$). Genotypes ECU-12967A and ECU-12967B showed the highest MGR, with rates of 0.77, and no statistical differences were observed between the application or non-application of GA₃. On the other hand, genotypes ECU-2237 and ECU-11994A showed an MGR of 0.73 and 0.72, respectively, with differences between treatments with and without the use of growth regulator (GA₃). Meanwhile, the ECU-2259 genotype just reached an MGR value of 0.71 (Figure 4A). pH level affected MGR ($p < 0.001$). pH levels 4.0, 5.8, 6.5, and 7.5 showed an average MGR of 0.75, while the MGR at pH 3.5 was 0.73 (Figure 4B).

Germination speed (GSP) was significantly influenced ($p < 0.001$) by the GA₃*genotype interaction. In general, all genotypes showed higher GSP when not treated with GA₃, except for ECU-11994a, where the GA₃ treatment was superior to its counterpart without GA₃. The highest GSP was observed in genotypes ECU-12967A and ECU-12967B

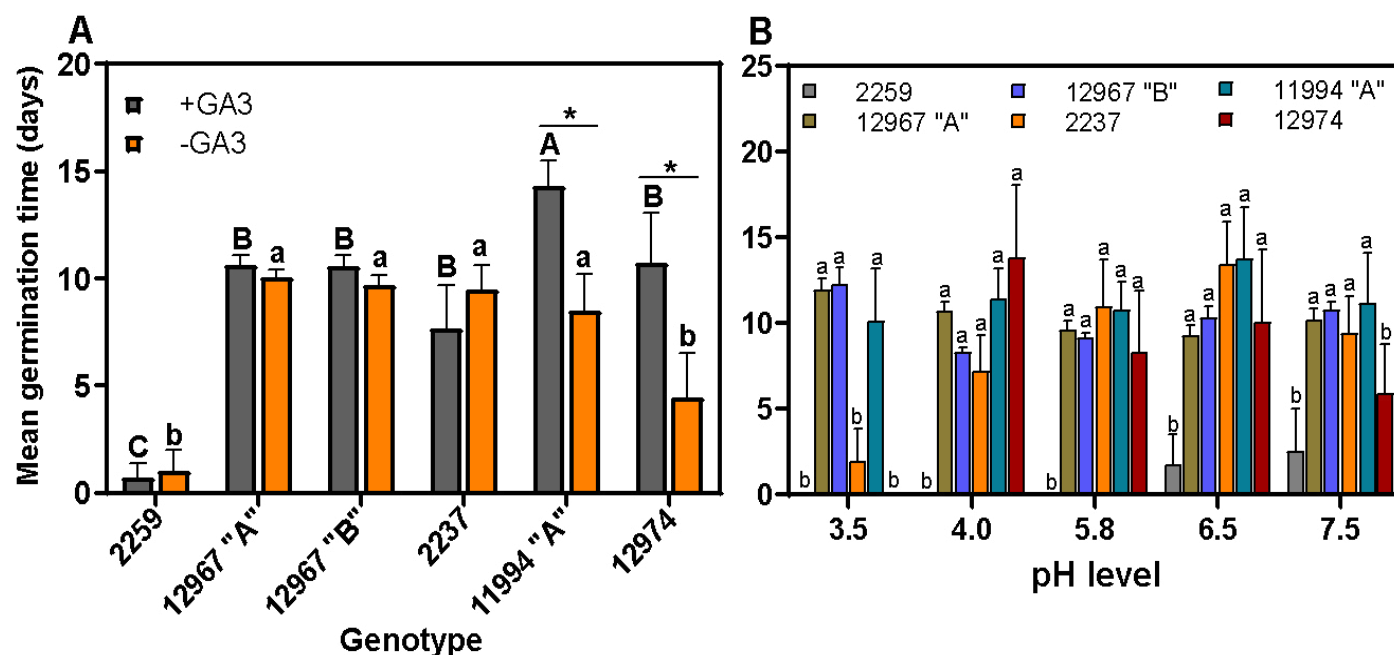


Figure 3: (A) Mean germination time (days) (MGT) of six *Capsicum* genotypes seeds with and without the addition of gibberellic acid (GA₃; 0.75 mg L^{-1}) under in vitro conditions. (B) Effect of pH levels on MGT of *Capsicum* spp. genotype seeds. Asterisks indicate statistical differences between genotypes treated or not with GA₃ according to the F test ($p \leq 0.05$). Identical letters within the same pH level do not differ statistically according to the Scott-Knott test ($p \leq 0.05$).

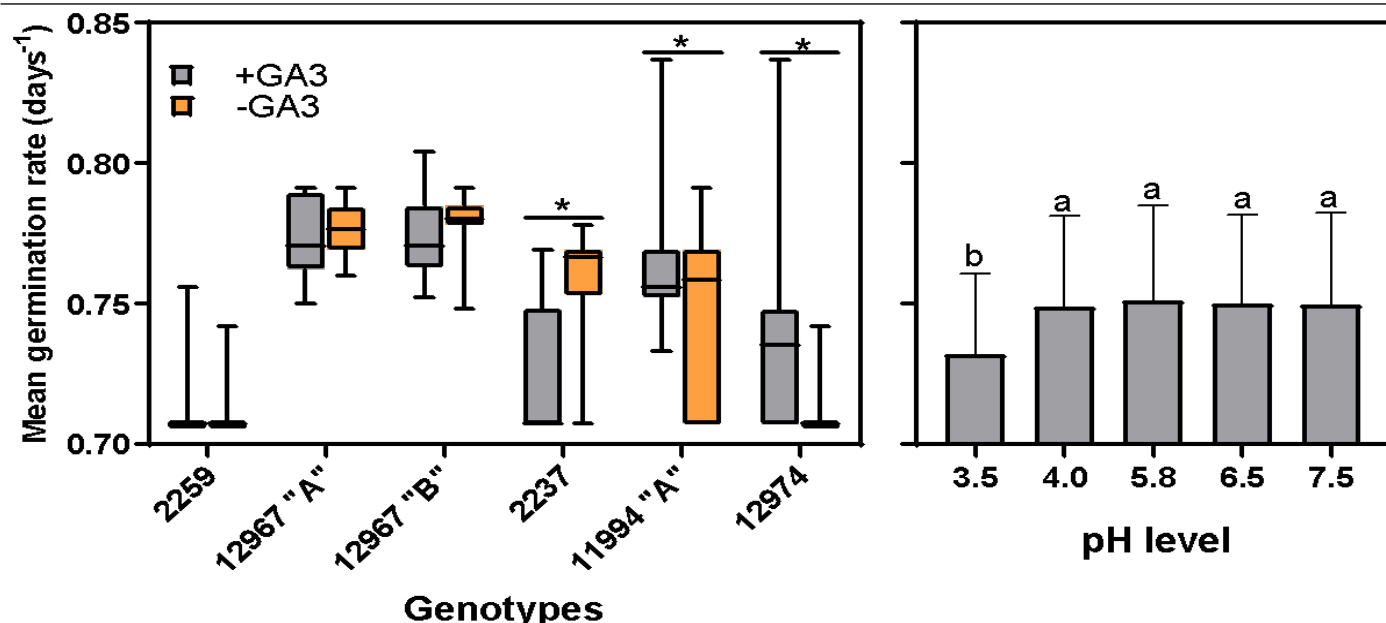


Figure 4: A) Mean germination rate (days⁻¹) (MGR) of six *Capsicum* genotypes seeds with and without the addition of gibberellic acid (GA₃; 0.75 mg L⁻¹) under in vitro conditions. B) Effect of pH levels on MGT for seeds of *Capsicum* spp. genotype. Asterisks indicate statistical differences between genotypes treated or not with GA₃ according to the F test ($p \leq 0.05$). Identical letters within the same pH level do not differ statistically according to the Scott-Knott test ($p \leq 0.05$).

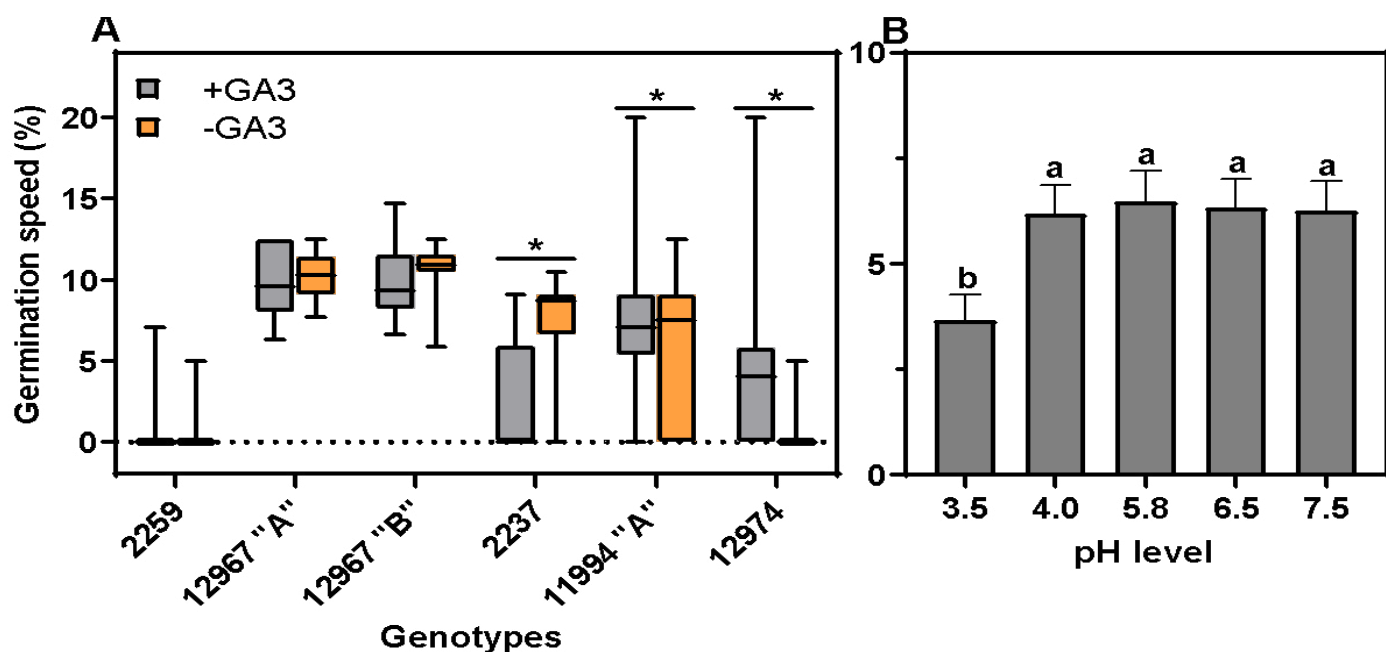


Figure 5: A) Germination speed (GSP) of six *Capsicum* genotypes seeds with and without the addition of gibberellic acid (GA₃; 0.75 mg L⁻¹) under in vitro conditions. B) Effect of pH levels on GSP of *Capsicum* spp. genotype seeds. Asterisks indicate statistical differences between genotypes treated or not with GA₃ according to the F test ($p \leq 0.05$). Identical letters within the same pH level do not differ statistically according to the Scott-Knott test ($p \leq 0.05$).

without GA₃ addition, with 9.82 and 9.92%, respectively. ECU-11994A showed a GSP of 8.02%, while genotypes ECU-2259 and ECU-12974 showed GSP < 4.23% (Figure 5A). On the other hand, the simple effect of the pH factor showed statistical differences ($p < 0.001$) among its levels. pH levels 4.0, 5.8, 6.5, and 7.5 showed an average GSP of 6.29%, statisti-

cally superior to pH 3.5, with a GSP value of 3.66% (Figure 5B).

Plantlet height at 60 days after establishment was significantly affected ($p < 0.01$) by the genotype*pH interaction. Plants grown at pH 3.5 showed no seedling growth, except for the ECU-12967A genotype,

which had an average height of 1.12 cm. The highest plant height was exhibited by genotypes ECU-12967A and ECU-12967B at pH 4.0, 5.8, and 6.5, with mean of 4.18, 4.37, and 4.75 cm, respectively (Table 3). Meanwhile, genotypes ECU-2237, ECU-11994A, and ECU-12974 showed mean of 2.53, 2.62, and 2.70 cm at pH levels 4.0, 5.8, and 6.5, respectively. At pH 7.5, genotypes ECU-12967, ECU-12968B, ECU-2237, and ECU-11994A presented an average plant height of 3.93 cm. Finally, the ECU-2259 genotype showed an average height of 0.34 cm considering all pH levels (Table 3).

Table 3: Effect of pH on plant height (cm) of six *Capsicum* genotypes growing under in vitro conditions.

Genotype	pH level				
	3.5 ^{ns}	4.0	5.8	6.5	7.5
ECU-2259	0.00	0.00c	0.00d	0.87c	0.87b
ECU-12967 ^a	1.12	4.12a	4.50 ^a	5.00a	4.50a
ECU-12967B	0.00	4.25a	4.25 ^a	4.50a	4.25a
ECU-2237	0.00	2.12b	2.88b	3.00b	3.25a
ECU-11994 ^a	0.00	3.00b	3.00b	3.00b	3.75a
ECU-12974	0.00	2.50b	2.00c	2.12b	1.75b
*C.V. (%)	60.43				

* Coefficient of variation; identical letters within the same column do not differ statistically according to the Scott-Knott test ($p \leq 0.05$); ^{ns} non-significant differences.

The number of leaves was influenced by the effect of the three factors studied: GA₃*genotype*pH. Seeds treated with GA₃ developed seedlings with a greater number of leaves compared to seedlings derived from untreated seeds. At treatment pH 3.5 with GA₃, only ECU-12967A showed a significant number of leaves

(4.75), while the other genotypes did not (Table 4). At pH 4.0 with GA₃ addition, genotypes ECU-12974, ECU-11994A, ECU-12967A, and ECU-12967B showed an average of 4.88 leaves, while ECU-2259 and ECU-2237 exhibited a mean of 0.50 leaves. In this same pH level (4.0), genotypes treated with GA₃ showed a greater number of leaves compared to seedlings without GA₃, except for ECU-2237 (Table 4).

Seedlings of ECU-11994A grown at pH 5.8 showed the highest number of leaves (6.50), while genotypes ECU-12967A, ECU-12967B, and ECU-12974 showed an average of 4.00 leaves/plant. Conversely, ECU-2259 did not show leaves. Except for ECU-2237, all genotypes showed a greater number of leaves when treated with GA₃ (Table 3). At pH 6.5, genotypes ECU-12967A, ECU-12967B, ECU-2237, ECU-11994A, and ECU-12974 showed an average of 4.15 leaves, significantly higher than the ECU-2259 with 0.75 leaves. Except for ECU-2237, all other genotypes showed a greater number of leaves when treated with GA₃ (Table 4).

Finally, genotypes ECU-12967A, ECU-12967B, ECU-11994A, and ECU-12974 showed a mean of 4.56 leaves/plant. Conversely, the lowest number of leaves was observed in ECU-2237 and ECU-2259 with 2.25 and 0.00 leaves, respectively. Except for ECU-2259 and ECU-2237, the rest of the genotypes showed a greater number of leaves when GA₃ was added to the culture medium.

Seed disinfection

The immersion of seeds in 70% ethanol for one minute has been widely recognized for its ability to

Table 4: Effect of pH on number of leaves/plant¹ of six *Capsicum* genotypes growing under in vitro conditions with and without growth regulator (gibberellic acid; GA₃).

Genotype	pH level									
	3.5		4.0		5.8		6.5		7.5	
	+GA ₃	-GA ₃ ^{ns}	+GA ₃	-GA ₃	+GA ₃	-GA ₃	+GA ₃	-GA ₃	+GA ₃	-GA ₃
ECU-2259	0.00b	0.00	0.00b	0.00c	0.00c	0.00c	0.75b	0.00b	0.00c	0.75c
ECU-12967 ^a	4.75a	0.00	3.50a	2.75b	3.75b	2.00b	4.25 ^a	2.50a	4.75a	2.00b
ECU-12967B	0.00b	0.00	5.00a	3.25b	4.50b	3.00b	4.25 ^a	2.50a	3.75a	2.25b
ECU-2237	0.00b	0.00	1.00b	5.50 ^a	1.50c	7.25a	4.00a	4.25a	2.25b	6.25a
ECU-11994 ^a	0.00b	0.00	5.25a	2.75b	6.50a	2.75b	4.25 ^a	3.00a	4.75a	3.00b
ECU-12974	0.00b	0.00	5.75a	1.25c	3.75b	0.50c	4.00a	0.50b	5.00a	0.00c
C.V. (%)	60.20									

* Coefficient of variation; identical letters within the same column do not differ statistically according to the Scott-Knott test ($p \leq 0.05$); ^{ns} non-significant differences.

eliminate efficiently surface microorganisms (George *et al.*, 2008). This treatment plays a crucial role in the initial reduction of microbial load, which can partly explain the low contamination rate observed in the present study. Besides, the treatment with 1% sodium hypochlorite (NaClO) for 15 minutes is a crucial step for eliminating more resistant pathogenic microorganisms, such as bacteria and fungi, which might persist after ethanol treatment (Yildiz *et al.*, 2012). The combination of these two disinfestation agents has proven to be highly effective in ensuring the sterility of plant explants, minimizing the risk of contamination during *in vitro* cultivation (Lima *et al.*, 2018). These findings support the importance of implementing robust and rigorous disinfestation protocols in the preparation of plant explants for *in vitro* culture, which is essential for guaranteeing the success and reproducibility of plant biotechnology experiments. Although disinfestation protocols are not always capable of eliminating microorganisms present in plant tissues (Martínez-Canto *et al.*, 2023), NaClO is one of the most widely used disinfecting agents in tissue culture for its effectiveness (Rojas *et al.*, 2020), which is reflected in the results observed in this study. Telci *et al.* (2011) reported seeds disinfection of *Lathyrus chrysanthus* Boiss. Under *in vitro* conditions, that seedlings grown from 3.75% NaClO sterilization for 15 min were more vital and well-grown.

Seed germination

The application of gibberellic acid (GA_3) showed a notable effect on the germination percentage, with a mean of 0.2708 and a mean germination rate of 0.5644 days⁻¹. Additionally, a significant decrease in the mean germination time was observed when GA_3 was applied, indicating an acceleration in the germination process. These findings support the germination-promoting function associated with GA_3 , corroborating previous research that highlights its role in regulating key physiological processes in seed germination (Smith and Weller, 2020). This is consistent with the reports by Eremrena and Mensah (2016), who determined that different plant growth regulators and nitrogenous compounds influence the germination rate and overall success of *Capsicum frutescens* L. seed germination. Furthermore, this phytohormone can break seed dormancy and replace environmental stimuli, acting in active growth areas, such as embryos, to favor germination (Hernández *et al.*, 2019; Mandujano *et al.*, 2007). The variation due to genotype was highly significant in all germination indices, underscoring the

genetic influence on seed germination capacity. This result highlights the importance of genetic selection in crop improvement programs to enhance germination and plant establishment. Additionally, a significant influence of pH level on germination parameters was observed, suggesting seed sensitivity to acidic or alkaline environmental conditions. These results align with previous studies demonstrating the influence of pH on various plant physiological processes, including seed germination (Mandić *et al.*, 2023; Kaya *et al.*, 2020).

The interaction between GA_3 and genotype was also significant in most germination indices, indicating a differential response to GA_3 treatment depending on the seed genotype. This interaction highlights the importance of considering genetic variability when applying growth regulators to improve germination in different crop varieties. This complex interaction indicates that multiple factors influence the germination process and underscores the importance of considering these components together to fully understand the observed variability in seed germination. The results show that, in general, *C. frutescens* seeds treated with GA_3 exhibited a higher germination percentage compared to untreated seeds, except for the ECU-2237 genotype. This exception might be related to specific genetic characteristics of the genotype that make it less receptive to GA_3 treatment. This finding is in line with previous research demonstrating variable responses of different plant genotypes to hormonal treatments (Pérez-Jiménez *et al.*, 2014).

Regarding pH influence, significant differences were observed among genotypes in their response to different pH levels. For example, genotypes ECU-12967A and ECU-12967B did not show differences in the evaluated pH levels, while the ECU-11994A genotype exhibited the highest germination at pH 4.0. Similar findings have been reported in studies investigating the effect of pH on seed germination, where specific genotypes show optimal germination rates at pH levels (Almansouri *et al.*, 2001; Baskin and Baskin, 2014). Gibberellic acid application significantly accelerated the germination process of *Capsicum* seeds, especially in seeds sown at pH 4.0, where an early onset of germination was observed four days after sowing. These results agree with previous studies demonstrating GA_3 's role in regulating seed germination and promoting initial seedling growth (Piskurewicz and López-Medina, 2009).

Results obtained in this *in vitro* study show a clear influence of the interaction between genotype and gibberellic acid (GA_3) on various parameters related to *Capsicum* seed germination. The use of GA_3 to break seed dormancy has been extensively documented in various plant species, as in the case of *Capsicum annuum*, where Mutalib *et al.* (2024) demonstrated that priming with GA_3 significantly enhances seed germination and root growth in seedlings. Specifically, it was observed that mean germination time (MGT) was significantly affected by this interaction ($p < 0.01$), highlighting the importance of considering both the genetic material of the plants and the use of growth regulators in germination protocols (Zaman *et al.*, 2015; Abbas *et al.*, 2020). For instance, ECU-11994A genotype exhibited the highest MGT, suggesting greater dormancy in these seeds compared to other genotypes, which can have practical implications in selecting planting material in breeding programs (Mahendran *et al.*, 2024).

Additionally, significant differences were observed in mean germination rate (MGR) and germination speed index (GSP) due to the interaction between genotype and GA_3 treatment ($p < 0.001$), indicating that the application of this growth regulator can modulate the speed and efficiency of germination in different *Capsicum* genotypes (Ahmed *et al.*, 2020). These results are consistent with previous studies demonstrating the key role of growth regulators in seed germination and initial seedling growth (Shu *et al.*, 2016). Moreover, the culture medium's pH showed a significant effect on MGR and GSP ($p < 0.001$), suggesting that the medium's acidity can influence the speed and efficiency of *Capsicum* seed germination (Lima *et al.*, 2018). These trial results are consistent with the literature highlighting the importance of medium pH in various plant physiological processes, including seed germination (Chen *et al.*, 2014). Germination was not affected by pH levels but influenced by the different accessions, which does not align with Rojas *et al.* (2020) in the case of *C. chinense* species, where higher germination was obtained in slightly acidic culture media. This might be due to specific genetic differences between the *C. frutescens* and *C. chinense* species.

The *in vitro* study reveals a significant interaction between genotype and medium pH on plantlet's height, 60 days after establishment. For example, it was found that plantlets grown in a medium with pH 3.5 barely

showed growth, except for the ECU-12967A genotype, suggesting a differential adaptation of certain genotypes to acidic medium conditions. Additionally, it was observed that GA_3 treatment influenced the number of leaves in plantlets, highlighting the role of growth regulators in plant morphogenesis. Specifically, it was found that at pH 3.5 with GA_3 treatment, only the ECU-12967A genotype presented a significant number of leaves, suggesting a specific genotype response to this hormonal treatment under acidic conditions. Furthermore, it was evident that the number of leaves was higher in genotypes treated with GA_3 compared to those not treated, underscoring the positive effect of this growth regulator on seedling foliar development.

Conclusions and Recommendations

The germination of *C. frutescens* is largely determined by the accession they belong to. Seeds of accessions 12967A and 12967B showed the highest germination percentages. The presence of GA_3 affected germination but did not influence seedling growth, while the different pH levels did not affect germination in these genotypes. Plantlets' height at different pH levels was determined by the characteristics of each accession. The tolerance to different pH levels of the 12970A and 12979B accessions is a promising characteristic that could result in a viable option for planting these cultivars in fields with different ranges of pH levels, which can help minimize losses in vegetative growth and yield parameters during production.

Novelty Statement

This study is the first to comprehensively evaluate the combined effects of pH levels and GA_3 on the germination and early seedling growth of *Capsicum frutescens* using multiple accessions. It highlights the resilience of specific *C. frutescens* genotypes (ECU-12967A and ECU-12967B) to varying pH conditions, providing valuable insights into their adaptability for *in vitro* propagation. The findings contribute to improving germination protocols for *C. frutescens*, enhancing its cultivation potential under diverse environmental conditions and offering practical solutions to reduce growth and yield losses due to suboptimal soil pH during production.

Author's Contribution

Bernardo Ganchozo-Zambrano: Conceptualization, investigation, methodology.

Genesis Bravo-Vélez: Conceptualization, investigation, methodology, writing.

Francisco Arteaga-Alcívar: Conceptualization, investigation, methodology, validation, writing – review & editing.

Liliana Corozo-Quiñónez: Conceptualization, investigation, methodology, validation, writing – review and editing.

Luis Alberto Saltos-Rezabala: Formal analysis, investigation, supervision, visualization, writing – original draft, writing – review & editing.

Fátima Macias Ponce: Conceptualization, investigation, methodology.

Álvaro Monteros-Altamirano: Conceptualization, writing-original draft, writing-review and editing, visualization.

Data availability

Data will be made available on request.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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