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SEED VIABILITY, GERMINATION, AND SEEDLING GROWTH OF CAPSICUM CULTIVATED SPECIES

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ABSTRACT

Seed viability of domesticated and cultivated *Capsicum* species: *Capsicum annuum* L., *Capsicum baccatum* L., *Capsicum chinense* Jacq., *Capsicum frutescens* L., and *Capsicum pubescens* Ruiz & Pav. was tested by the tetrazolium method. The seeds were soaked in three tetrazolium salt solutions of 1, 0.5 and 0.2%, and temperature conditions of 30 - 35 °C for 3 h. The percentage of seeds stained in relation to the total number of seeds was evaluated. To estimate germination, the seeds were subjected to chemical treatments with gibberellic acid (GA_3) at doses of 0, 100, 200, 300, and 400 mg L^{-1} at room temperature for 24 h. The germination rate was estimated, while plant height was evaluated at 10, 20 and, 30 days after sowing (DAS). *Capsicum* seed viability fluctuated between 52.44% and 97.11%. The highest percentage of viability was obtained with the 1% dose of tetrazolium (81.63%), while the 0.2% dose resulted in the lowest average value (63.7%). In addition, *C. annuum*, *C. baccatum* and *C. chinense* showed high staining patterns, while most of the observed patterns in *C. frutescens* corresponded to non-viable seeds. After GA_3 application, average seed germination was 100% in *C. chinense*; 75.6% in *C. baccatum*; 73.32% in *C. annuum*; and 61.5% in *C. frutescens*. However, the application of GA_3 did not have an additional effect on the germination of *Capsicum* seeds. The highest plant height at 30 DAS was observed in accessions of *C. annuum* (9.08 cm), while *C. chinense* presented the lowest value (4.49 cm).

Keywords: Germplasm bank, gibberellic acid, *Capsicum annuum*, *C. baccatum*, *C. chinense*, *C. frutescens.*

INTRODUCTION

Species of the genus *Capsicum* L. (Solanaceae) commonly known as peppers or chilies (Liu et al., 2017), are native to tropical America (Carrizo -García et al., 2016), being widely consumed worldwide (Ramchiary and Cole, 2019). The genus consists of 42 species (Bánki et al., 2022), five of which have been classified as domesticated and cultivated (*Capsicum annuum* L., *Capsicum baccatum* L., *Capsicum chinense* Jacq., *Capsicum frutescens* L., and *Capsicum pubescens* Ruiz & Pav), which are used both in food and in the industry (Tripodi and Kumar, 2019).

Seed vigor is a complex trait that encompasses aging tolerance, seed dormancy, viability, rapid germination, and seedling establishment, especially in suboptimal conditions (Bewley and Black, 2013). The quality of seeds of the cultivated species of *Capsicum* is an important factor in its commercial value, and thus there is a need to produce seeds with greater germination capacity and slow deterioration in storage (Siri et al., 2013). In addition, rapid seed germination and seedling emergence is essential for a successful establishment of *Capsicum* species (Ozbay, 2018).

Germination is a physiological process that begins with the absorption of water by the seed (imbibition) and culminates with the emergence of the embryo from its surrounding covers. However, in many occasions, the seeds are not able to germinate after maturation and dispersal, either because they are dormant or because the environmental conditions are not favourable (Bewley and Black, 2013). Additionally, seeds that undergo prolonged aging eventually lose their viability (ability to germinate) completely, which largely depends on environmental storage conditions, seed genetics, and maternal environment (Bewley and Black, 2013).

In some *Capsicum* species, germination percentage is associated with domestication (Pickersgill, 2016), which has been a barrier to their cultivation. For instance, wild populations of *Capsicum annuum* var. *glabriusculum* have a germination rate of 2-50%, while that of cultivated populations is 70% (González-Jara et al., 2011; Sandoval-Rangel et al., 2018).

Seed viability indicator tests include physical and physiological variables of the seeds (Milošević et al., 2010). The tetrazolium test indirectly determines the respiratory activity in the cells in seed tissues (França-Neto and Krzyzanowski, 2019), and it has been used in several plant species (Belniaki et al., 2020; Salazar-Mercado et al., 2020). The advantage of this technique is the accuracy and speed in estimating the vigor and viability of seeds in a short time (Alves et al., 2006; Dias and Alves, 2008). For *C. annuum*, it has been described that tetrazolium can estimate growth based on color intensity (Kusumawardana and Pujiasmanto, 2018), and thus the method could be useful for other species and varieties of the genus *Capsicum*.

Viable seeds are necessary for germination, but germination is also controlled by endogenous factors of the seed (phytohormones) and external factors such as light, humidity, and temperature (Ravindran and Kumar, 2019). Chemical, physical, and biological pre-germination treatments can be used to provide the right conditions and accelerate seed germination

(Srivastava et al., 2021). Gibberellic acid (GA_3) has been identified as a critical hormone in the regulation of seed germination by counteracting the inhibition imposed by abscisic acid (ABA). Consequently, the increase in GA_3 concentration after imbibition is essential for the rupture of the testa and the endosperm, and the subsequent emergence of the radicle (Ravindran and Kumar, 2019). In this sense, Tripathi et al. (2022) have mentioned that these growth regulators also control other important aspects in plant development.

In the case of *Capsicum annuum* var. *glabriusculum*, it was determined that there are no morphological or physical barriers that prevent imbibition and proper germination of the seeds, being explained by a phenomenon of physiological latency that could be broken with germinative pre-treatments such as the exogenous application of GA_3 (Cano-Vázquez et al., 2015).

The research objectives were: to evaluate the viability of the seeds of five *Capsicum* species stored for more than three years, and to determine the effect of gibberellic acid on the germination capacity and growth of *Capsicum* seedlings.

MATERIAL AND METHODS

Seeds

Five Ecuadorian seed accessions from the genebank of the National Institute of Agricultural Research - INIAP were evaluated for each cultivated species: *C. chinense*, *C. baccatum* and *C. frutescens* (Table 1). These accessions were collected in the 4 natural regions of Ecuador (Coast, Andes, Amazonia, and Galapagos). Only in the case of *C. annuum*, three accessions were evaluated, two from collections made in home gardens of Santo Domingo parish, province of Esmeraldas (Coastal region).

Seed viability test

To determine the viability of *Capsicum* spp. seeds, the tetrazolium (2, 3, 5 Triphenyl tetrazolium chloride) staining test was performed (ISTA, 2022). A completely randomized design (CRD) with a factorial arrangement of 20 (accessions) x 3 (tetrazolium dose: 1%, 0.5%, and 0.2%), with four repetitions, was used.

For each dose of tetrazolium, 500 seeds per species (100 seeds/accession) were placed in distilled water for 24 h for hydration, to initiate

Table 1. *Capsicum* spp. accessions obtained from the INIAP's GenBank evaluated in this study.

	Species	Code	Province	Altitude (masl)	Latitude	Longitude
1	*C. annuum*	ECU-2254a	El Oro	410	03.46S	80.01W
2	*C. annuum*	ECU-2254b	El Oro	410	03.46S	80.01W
3	*C. annuum*	ECU-2255	Loja	1700	04.13S	79.26W
4	*C. annuum*	LCQ-A*	Esmeraldas	100	01.10S	80.17W
5	*C. annuum*	LCQ-A3*	Esmeraldas	100	01.10S	80.17W
6	*C. baccatum*	ECU-2231	Cotopaxi	2600	00.55S	79.36W
7	*C. baccatum*	ECU-12840	Loja	1710	04.03.711 S	79.39.283 W
8	*C. baccatum*	ECU-12846	Loja	1640	04.03.287 S	79.38.502 W
9	*C. baccatum*	ECU-12859	Loja	1650	04.21.984 S	79.10.600 W
10	*C. baccatum*	ECU-12978	Morona S.	1625	02.39.448 S	78.12.471 W
11	*C. chinense*	ECU-2239b	Manabí	100	01.10S	80.17W
12	*C. chinense*	ECU-2241	Manabí	90	01.10S	80.45W
13	*C. chinense*	ECU-2256	Galápagos	5	00.58S	91.00W
14	*C. chinense*	ECU-11996	Morona S.	1040	2.21.065 S	78.09.314W
15	*C. chinense*	ECU-12979	Morona S.	700	02.34.829 S	78.10.636 W
16	*C. frutescens*	ECU-2246	El Oro	420	03.46S	80.01W
17	*C. frutescens*	ECU-2251	El Oro	410	03.46S	80.01W
18	*C. frutescens*	ECU-2259	Loja	1200	04.24S	79.28W
19	*C. frutescens*	ECU-12968	Morona S.	850	03.31.038 S	78.32.703 W
20	*C. frutescens*	ECU-12970	Morona S.	1125	03.22.076 S	78.33.540 W

Accessions from INIAP´s Ecuadorian genebank. *New collected accessions, from Santo Domingo parish, province of Esmeraldas.

the activity of the dehydrogenase enzymes and soften the seeds. Subsequently, longitudinal cuts were made with a scalpel to expose the cotyledons. They were then placed in a 13 × 100 mm test tube, and the solution of 2, 3, 5 Triphenyl tetrazolium chloride (TZ) was added until the seeds were covered. Subsequently, the test tubes were wrapped in aluminum foil to avoid direct light and placed in an oven at 30-35 °C for 3 h. The stained seeds were evaluated using the scale proposed by Sandoval-Rangel et al. (2018) and observed in a stereoscope (Carl Zeiss brand, STEMI 305 model). The percentage of stained seeds was calculated using the following formula: [(Number of seeds stained red) / (Total number of seeds)] x 100.

Seed viability was classified according to the staining pattern of Kusumawardana and Pujiasmanto (2018) with modifications, which considers that seeds are viable when the embryos and cotyledons show a bright red color, whereas seeds are non-viable when the embryos and cotyledons show a white color.

Seed germination and plantlet growth

To evaluate *Capsicum* spp. germination rate, the methodology proposed by De la Rosa et al. (2012) was followed. A completely randomized design (CRD) was used with a factorial arrangement of 20 (accessions) x 5 (GA_3 dose: 0, 100, 200, 300 and 400 mg L^{-1}), with four repetitions. For each dose, 10 seeds of each accession were used. The seeds were washed with sterile distilled water and dried at room temperature. Subsequently, they were placed in Petri dishes and submerged in different treatments with GA_3, at room temperature for 24 h. Before sowing, the seeds of each accession and treatment were dried and planted in trays of 50 alveoli (dimensions: 55 cm long, 29 cm wide, and 7.5 cm high), containing peat as substrate. To evaluate the germination percentage of the seeds, the emergence of the hypocotyl was considered at 16 days after sowing (DAS), and the following formula was applied:

GER=SG/TSE x 100,
where: GER: germination percentage,
SG: seeds germinated and,
TSE: total seeds evaluated.

The plant height (PH) was measured at 10, 20 and 30 DAS.

Before performing the analysis of variance (ANOVA) in each variable, the normality of residuals (Shapiro-Wilk test) and homogeneity of variances (Bartlett's test) were verified. When there was statistical significance, mean comparisons were performed using the Scott-Knott test (P < 0.05). In some cases, where the variables did not meet the assumptions of the ANOVA, a nonparametric analysis by aligned ranks transformation ANOVA (ART ANOVA) was performed, followed by the Sidak test for the comparison of means. The principal component analysis (PCA) was used. The association among all quantitative variables was performed using Spearman's rank correlation (P < 0.05). Data were analyzed using the free R Development Core Team (2022) Version 4.2.2.

RESULTS AND DISCUSSION

Significant differences (P< 0.05) were found in accessions, doses, and interaction (Accesions x Doses) in the species *C. baccatum*, *C. chinense* and *C. frutescens*, while *C. annuum* recorded differences only in the interaction. Regarding species, *C,annuum* recorded 74,67% of viability; for accession, LCQ-A recorded the highest percentage (88.13 %), while ECU-2255 accesion had the lowest value (54.63). In *C. baccatum*, viability reached an average value of 74.33%, fluctuating between 97 and 40%; the highest value was recorded in ECU-12846 and the lowest in ECU-2231. In *C. chinense*, four of the five accessions had viability percentages greater than 90% (ECU-2241, ECU-11996, ECU-2256 and ECU-2239b). On average, this species presented the highest significant value (92.91%), while *C. frutescens* presented the lowest value (52.45%) (Table 2).

Capsicum species responded differently indicating that viability might be related to seed storage time (more than three years in all accessions). Furthermore, it has been described that the intrinsic genetic characteristics influence the viability capacity over time (Salazar-Mercado et al., 2020). About doses of tetrazolium, the 1% dose presented the highest percentages of viability in all species, while the 0.2% dose presented the lowest percentages. This shows that the use of high concentrations of tetrazolium is preferable in the *Capsicum* genus as it occurs in other species (Mercado and Jaimes, 2022). Tetrazolium salts generates water-soluble sulfonated formazans, which are rapidly absorbed by cells and their concentration is directly proportional to the number of living cells in the seed embryo (Stockert et al., 2018). Lower concentrations of tetrazolium (e.g., 0.2%), did not generate enough sulfonated formazans required to evaluate viability in *Capsicum* seeds. Salazar-Mercado et al. (2020), exposed *C. annuum* seeds to two concentrations of tetrazolium (1% and 0.5%) and exposure times (24 and 48 h) and obtained statistically significant differences (P< 0.05) at a

Table 2. Effect of different doses of tetrazolium on the viability of *Capsicum* spp. seeds.

Accessions	Species	Tetrazolium dose 1%	0.5%	0.2%
ECU-11996	*C. chinense*	98.0 ± 1.2 aA	95.3 ± 0.9 bA	84.0 ± 2.1 bB
ECU-12840	*C. baccatum*	65.0 ± 2.9 dA	56.3 ± 0.9 eA	46.7 ± 3.3 eB
ECU-12846	*C. baccatum*	96.0 ± 3.3 aA	94.3 ± 1.2 bA	84.0 ± 2.1 bB
ECU-2231	*C. baccatum*	64.0 ± 2.1 dA	59.3 ± 0.9 eA	40.0 ± 5.8eB
ECU-12859	*C. baccatum*	97.0 ± 0.6 aA	95.0 ± 0.6 bA	75.0 ± 1.7 cB
ECU-12968	*C. frutescens*	51.3 ± 0.9 eA	42.7 ± 0.9 fA	27.7 ± 1.8 fB
ECU-12970	*C. frutescens*	85.7 ± 3.0 cA	86.7 ± 0.9cA	74.3 ± 2.3 cB
ECU-12978	*C. baccatum*	84.7 ± 2.9 cA	92.7 ± 0.9 bB	65.0 ± 2.9 dC
ECU-12979	*C. chinense*	94.0 ± 2.1 aB	94.0 ± 0.6 bA	78.3 ± 5.6 cB
ECU-2239b	*C. chinense*	99.7 ± 0.3 aA	91.3 ± 1.9 bB	88.0 ± 4.0 bB
ECU-2241	*C. chinense*	98.3 ± 0.9 aA	89.0 ± 0.6 cB	92.3 ± 2.9 aB
ECU-2246	*C. frutescens*	90.0 ± 0.6 bA	78.3 ± 1.2 dB	52.7 ± 3.3 eC
ECU-2251	*C. frutescens*	27.3 ± 4.3 fA	30.7 ± 4.1 gA	18.0 ± 7.0 fB
ECU-2254a	*C. annuum*	92.3 ± 1.5 bA	88.0 ± 1.0 cA	78.3 ± 4.4 cB
ECU-2254b	*C. annuum*	64.3 ± 2.3 dA	52.3 ± 1.2 eB	47.3 ± 5.0 eB
ECU-2255	*C. annuum*	87.7 ± 0.9 cA	76.0 ± 3.1 dB	65.0 ± 2.9 dC
ECU-2256	*C. chinense*	98.3 ± 0.9 aA	98.3 ± 0.9 aA	94.7 ± 1.5 aB
ECU-2259	*C. frutescens*	56.7 ± 2.6 eA	40.0 ± 4.0 fB	24.7 ± 8.4 fC
LCQ-A	*C. annuum*	97.7 ± 2.9 aA	92.0 ± 3.2 bB	74.7 ± 3.3 cC
LCQ-A3	*C. annuum*	74.7 ± 0.3 dA	66.3 ± 0.6 eA	63.3 ± 2.6 dB

Means followed by the same letters do not differ, by the test of Scott-Knott, at 5% probability. Lower case letters in each column represent differences between accessions, and capital letters in the row indicate differences between tetrazolium doses. The analysis of variance was performed with the transformed arc sine square root of the primary values. Mean ± standard error of mean.

exposure time of 48 h, reporting viability of 99%; whereas the rest of the treatments (1%, 24 h and 0.5%, 24 h), showed no statistical differences between them, with viability percentages of 89% and 91%, respectively.

The tetrazolium test is a widely used method to assess seed viability and vigor. It is based on the reduction of a colorless salt (2,3,5-triphenyl tetrazolium chloride, TTC) to a red compound (formazan) by the dehydrogenase enzymes present in living tissues (Lakon, 1949; Moore, 1973). The test can reveal different patterns and intensities of staining in seed embryos, which can be related to their physiological status and potential germination performance (Copeland and McDonald, 2001). In this study, we observed diverse tetrazolium staining patterns across *Capsicum* accessions (Fig. 1), with *C. frutescens* showing predominantly non-viable seeds, as indicated by the absence or weak presence of formazan.

This suggests a possible effect of the tetrazolium test on the germination dynamics of this species, which deserves further investigation. On the other hand, the high staining levels in *C. annuum*, *C. baccatum*, and *C. chinense* indicate strong seed viability, in contrast to the difficulties observed in *C. frutescens*. The prevalence of non-viable seeds in *C. frutescens* may reflect underlying problems affecting germination success, possibly related to seed quality or physiological factors. For instance, some studies have reported that seed coat impermeability, dormancy, and low vigor are common causes of low germination rates in *Capsicum* seeds (Rami and Patel, 2014). Therefore, it is essential to understand the relationship between tetrazolium staining and germination performance in *C. frutescens* in order to develop specific strategies to enhance seed viability and increase overall germination rates. Knowledge about such relationship contributes to the broader discussion regarding the optimization of seed management practices for better conservation and utilization of *Capsicum* genetic resources.

The following staining colorations were observed: carmine red or intense pink (viable), white or yellowish white (not viable), and pale yellowish pink (not viable). This last coloration was considered non-viable, bearing in mind a history of other species such as Araucaria

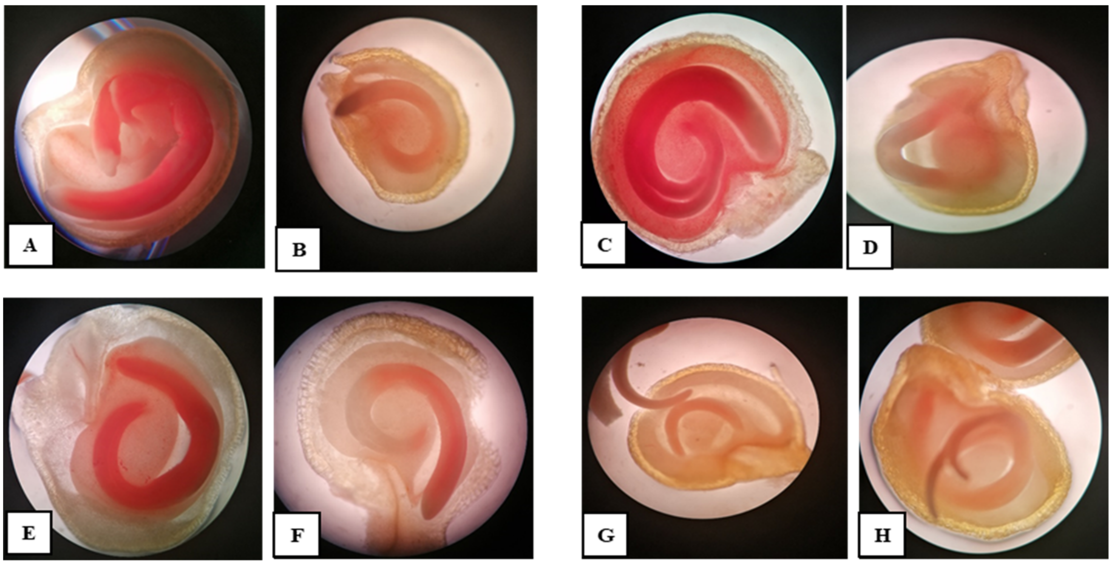

Fig. 1. Topographic pattern of tetrazolium in cultivated species of the genus *Capsicum*. A) *C. annuum* seeds with average staining pattern. B) *C. annuum* seeds with low staining pattern. C) *C. baccatum* seeds with a high staining pattern. D) *C. baccatum* seeds with low staining pattern. E) *C. chinense* seeds with a high staining pattern. F) *C. chinense* seeds with low staining pattern. G and H) *C. frutescens* seeds with low staining pattern.

angustifolia (Ariane et al., 2016), Glycine max (Salazar-Mercado and Botello-Delgado, 2018) and *Epidendrum* sp., (Salazar-Merado et al., 2020).

In the genus *Capsicum*, viability evaluations have only been carried out in *C. annuum*. For example, Martínez et al. (2019) reported viability percentage ranging from 78 to 96%, and well-stained cotyledons and embryos treated in a solution of 2,3,5-triphenyltetrazolium chloride (1%, w/v). For the rest of the cultivated species, reports are scarce.

Effect of gibberellic acid on seed germination capacity and seedling growth

The statistical analysis showed significant differences between for all the accessions. Seed germination fluctuated between 46.6 and 87% in *C. annuum*; between 40.30 and 89.75% in *C. baccatum*; between 42.15 and 91.95% in *C. frutescens*; and reached 100% in *C. chinense* (Table 3). The low percentage of viability (52%) observed in *C. frutescens* seeds may be due to several factors, such as seed coat impermeability, dormancy, and low vigor, which are common causes of low germination rates in *Capsicum* seeds (Ozbay, 2018; Adebisi and Abdul-Rafiu, 2016). GA_3 is a plant growth regulator that can enhance the physiological potential of seeds by stimulating the synthesis of enzymes involved in the mobilization of reserves and the degradation of the seed coat (Jaiswal et al., 2021; Mustafa et al., 2019).

In the present study, the application of GA_3 at varying concentrations significantly augmented the germination percentage and speed of seeds derived from diverse accessions of *C. frutescens*. Furthermore, there was a positive impact on the growth and development of the seedlings. It has been described that the optimal concentration and timing of GA_3 application may vary depending on the cultivar and environmental conditions (Ozbay, 2018). Therefore, further research is needed to optimize the use of GA_3 for enhancing the germination and seedling vigor of *C. frutescens*.

Other studies have also indicated that the application of different GA_3 concentrations has no effect on seed germination (Pico-Mendoza et al., 2020). However, Saldivar-Iglesias et al. (2010) found that higher concentration of GA_3 increased the germination percentage (87%) in *Jaltomata procumbens* (Cav.) J. L. Gentry with 250 mg L^{-1} of GA_3. Similarly, Eisvand et al. (2015) conducted a study on carrot seeds and, reported an increased germination rate with the use of GA_3 and salicylic acid (100 ppm). In fact, gibberellins stimulate hydrolysis enzymes, especially α-mylase, triggering seed germination (Tombegavani et al., 2020). Other trials have used sodium chloride (NaCl) combined with GA_3 (350 mgL^{-1}), obtaining up to 91.7 % germination

Table 3. Germination percentage and plant height (cm) in *Capsicum* spp. at 10, 20 and 30 days after sowing (DAS).

Accessions	Species	Germination (%)	Plant height (cm) at different days after sowing (DAS)		
			10 DAS	20 DAS	30 DAS
ECU-2251	*C. frutescens*	50.6 ± 1.8 a	0.59 ± 0.2 a	2.28 ± 0.1 a	3.13 ± 0.1 a
ECU-12968	*C. frutescens*	44.3 ± 5.4 ab	1.95 ± 0.1 a	3.60 ± 0.1 bcd	4.73 ± 0.1 b
ECU-2246	*C. frutescens*	68 ± 4.5 bcd	2.06 ± 0.1 a	4.63 ± 0.2 efg	6.71 ± 0.3 cd
ECU-2259	*C. frutescens*	52.4 ± 9.4 bc	2.08 ± 0.1 a	4.32 ± 0.3 def	6.17 ± 0.3 cd
ECU-12970	*C. frutescens*	92.4 ± 2.6 gh	3.02 ± 0.1 b	5.95 ± 0.2 i-l	8.33 ± 0.3 e
ECU-12979	*C. chinense*	100 ± 0.0 h	3.02 ± 0.1 b	3.28 ± 0.1 ab	4.30 ± 0.2 ab
LCQ-A3	*C. annuum*	71 ± 4. 0 b-e	3.03 ± 0.1 bc	5.59 ± 0.1 h-k	6.21 ± 0.1 cd
ECU-12978	*C. baccatum*	83 ± 5.1 efg	3.12 ± 0.1 bc	4.80 ± 0.1 fgh	5.91 ± 0.1 c
ECU-11996	*C. chinense*	100 ± 0.0 h	3.24 ± 0.1 bcd	3.47 ± 0.1 abc	4.27 ± 0.1 ab
ECU-2256	*C. chinense*	100 ± 0.0 h	3.34 ± 0.1 b-e	3.71 ± 0.1 bcd	4.31 ± 0.2 ab
ECU-12840	*C. baccatum*	40 ± 6.5 ab	3.46 ± 0.5 b-f	7.0 ± 0.7 j-m	7.94 ± 0.7 de
ECU-2231	*C. baccatum*	70 ± 5.9 c-f	3.56 ± 0.4 c-g	5.65 ± 0.4 g-j	6.68 ± 0.4 cd
ECU-2241	*C. chinense*	100 ± 0.0 h	3.66 ± 0.1 d-g	4.06 ± 0.1 cde	4.88 ± 0.2 b
ECU-2239	*C. chinense*	100 ± 0.0 h	3.80 ± 0.1 e-h	4.05 ± 0.1 de	4.48 ± 0.1 b
ECU-2254b	*C. annuum*	46 ± 3.7 ab	3.96 ± 0.2 e-h	7.09 ± 0.5 klm	8.46 ± 0.5 e
ECU-12859	*C. baccatum*	95 ± 2.5 gh	3.99 ± 0.1 fgh	5.01 ± 0.2 fhi	6.05 ± 0.2 c
ECU-2255	*C. annuum*	76 ± 2.7 cde	4.20 ± 0.1 ghi	7.10 ± 0.4 klm	8.52 ± 0.4 e
ECU-12846	*C. baccatum*	90 ± 3.8 fg	4.65 ± 0.1 hij	7.36 ± 0.3 lm	8.32 ± 0.3 e
ECU-2254a	*C. annuum*	86 ± 2.2 d-g	5.62 ± 0.2 ij	8.94 ± 0.3 mn	9.64 ± 0.3 ef
LCQ-A	*C. annuum*	87.5 ± 2.6 d-g	6.23 ± 0.1 j	10.87 ± 0.3 n	12.31 ± 0.3 f

Means followed by the same letters do not differ, by Sidak's multiple comparisons test, at 5% probability. The analysis of variance was performed by nonparametric Aligned ranks transformation ANOVA (ART anova). Mean ± standard error of mean.

in relation to the treatment without NaCl in *C. annuum* (De la Rosa et al., 2012).

Plants growth was progressive, in *C. annuum*, *C. baccatum*, and *C. frutescens* at 10 DAS, showing noticeable differences regarding plant height, while the growth was slower in *C. chinense* (Table 3). A study conducted by Lizarde et al. (2011) reported a plant height of 17 cm in Chiltepín (*Capsicum annuum* L. var. *glabriusculum*) plants after two months, and concluded that plant growth was carried out proportionally during the 8 weeks period, showing that GA_3 stimulates plant growth.

Plant height is an indicator of performance in terms of plant growth in the field. In our study, the high genetic variability of the evaluated accessions and their origin have a direct influence on this variable. Oh and Kim (2014) found that GA_3 applications at a concentration of 100 mg/L increased petiole elongation and plant height in *Cyclamen persicum*.

The principal component analysis (PCA) shows that *C. chinense* accessions presented higher viability and seed germination, with values of 92.4% and 100%, respectively. In contrast *C. frutescens* recorded the lowest values of 52.4% and 52.5% for the same parameters respectively. In relation to plant height, *C. annuum*, showed greater values, standing out the accession ECU-2254a and LCQ-A (9.64 and 12.31 cm, respectively) at 30 DAS, whereas *C. chinense* recorded a lower value of 4.4 cm (Fig. 2). The good performance of *C. annuum* accessions could be related to the adaptability to the different environments presented by the varieties and hybrids of *C. annuum*, which is the most planted species worldwide. Mireles-Rodriguez et al. (2015) and Cano-Vázquez et al. (2015) reported higher germination rates and an increase in the initial and final growth of plants of *Capsicum annuum* L. (var. *glabriusculum*) with 5000 ppm of GA_3. Likewise, Hernández et al. (2019) found that the height of *Capsicum annuum* L. (cv. 'Papri King) seedlings was greater in treatments with doses of 1 mg L^{-1} of GA_3. Furthermore, Torres and De Souza (2013) applied gibberellins (50 mg/L) in

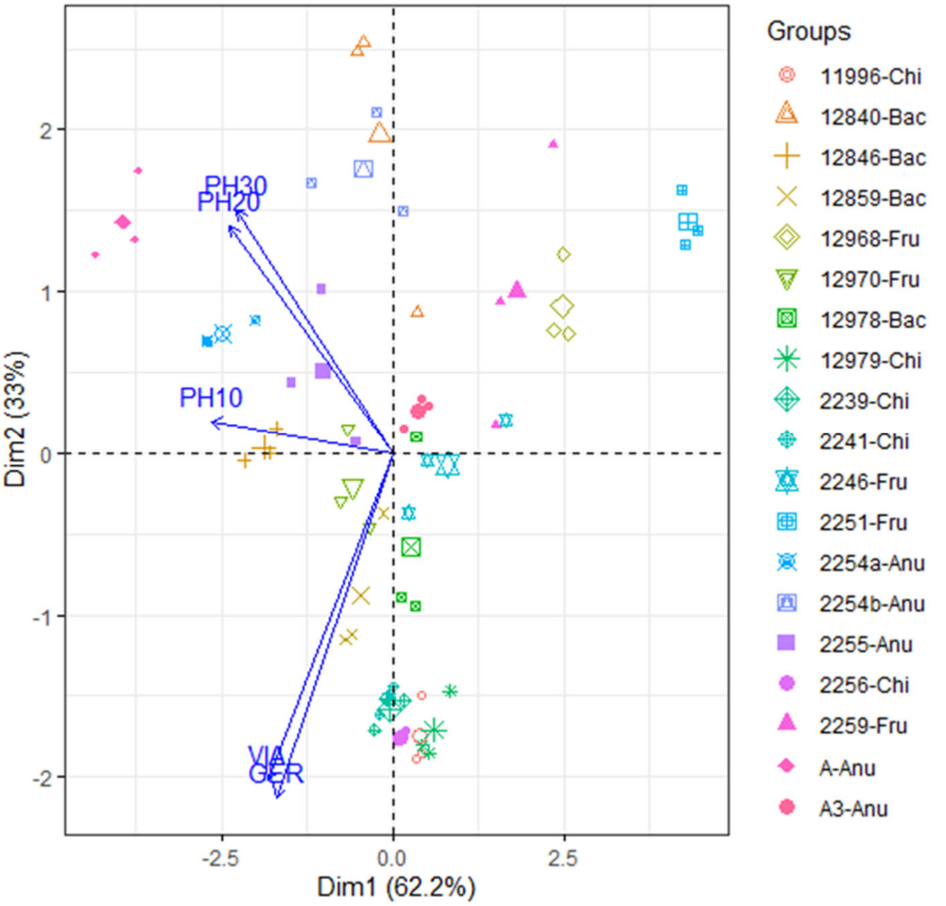

Fig. 2. PCA of variables: viability (VIA) and germination of seeds (GER), plant height (PH) measured at 10, 20 and 30 days after sowing in *Capsicum* accessions.

Capsicum frutescens, achieving 15 cm in height 30 days after germination.

Seed the viability and germination are key factors for the success of plant propagation, especially for increasing the production of threatened species or species of agronomic interest. Seed germination is influenced by various factors, such as temperature, humidity, substrate and storage time. Some *Capsicum* species exhibit dormancy, which is a delay or inhibition of germination, that can be overcome by pre-germination treatments, such as scarification, stratification, or the application of growth regulators (Elizalde et al., 2017).

Fig. 3 shows the correlation between the variables evaluated in terms of seed viability and germination of the cultivated species of *Capsicum*. There is a positive relationship between viability and germination (r_s = 0.86***), indicating that the higher the viability, the higher the germination. In addition, significant associations arose between plant height (10 DAS) and seed viability and germination as suggested by Spearman's correlation coefficients of 0.39 and 0.30. This was observed in all the evaluations conducted in the study, showing that the development of the plant depends on its initial growth.

This implies that seed quality, as determined by the tetrazolium test, has an effect on the initial growth and development of the seedlings. Therefore, the use of the tetrazolium test is recommended as a rapid and reliable method to assess the viability of *Capsicum* seeds, and to select the best seeds for propagation. However, the results also revealed that there are differences among the species of *Capsicum*, with *C. chinense* exhibiting the highest values of seed viability and germination, and *C. frutescens* recording the lowest. These differences may be due to genetic, physiological, or environmental factors that affect the seed quality and performance of

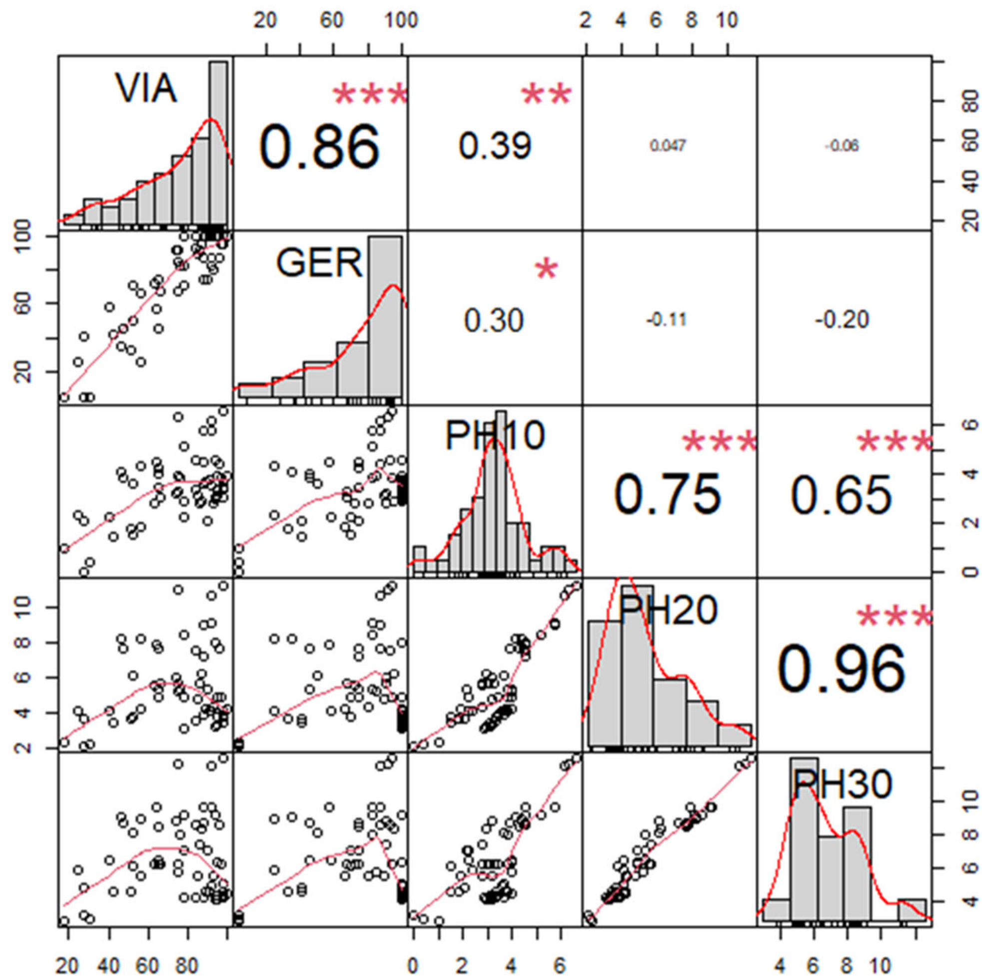

Fig. 3 Spearman correlation analysis of variables: viability (VIA) and germination of seeds (GER), plant height (PH) measured in 10, 20 and 30 days after sowing in *Capsicum* accessions.

each species. For example, some studies have reported that *C. chinense* has a higher tolerance to salinity and drought stress compared to *C. annuum* and *C. frutescens*, which may explain its better germination response (da Silva et al., 2019). Other studies have suggested that *C. frutescens* has a lower seed vigor and a higher dormancy level, which may account for its lower germination rate (Yamamoto and Nawata, 2006).

Therefore, the results of this study suggest that the propagation of *Capsicum* species requires the adjustment of protocols according to the specific characteristics and requirements of each species. Further studies are needed to determine optimal conditions and treatments, such as temperature, light, moisture, sowing depth, and pre-sowing treatments, can enhance the germination and growth of *Capsicum* seeds and seedlings.

CONCLUSIONS

The viability of *Capsicum* seeds that have been stored for intermediate periods of time is determined by the species. The seeds that maintain the highest viability are *C. chinense*, followed by *C. baccatum*, *C. annuum* and *C. frutescens*. The presence of GA_3 during the germination of *Capsicum* spp. seeds does not promote seedling growth, being specifically determined by the genetic characteristics of each accession.

LITERATURE CITED

Adebisi, M., and A. Abdul-Rafiu. 2016. Storage life of cayenne pepper (*Capsicum frutescens*) seeds following three drying methods. Seed Technology 33-42.

Alves, C., M. Malavasi, C. Zucareli, and U. Malavasi. 2006. Aplicação do teste de tetrazólio em sementes de *Gleditschia amorphoides* Taub. caesalpinaceae. Revista Brasileira de Sementes 28(3):101–107. doi: 10.1590/S0101-31222006000300015

Ariane, B., J. Nogueira, E. Serra, and N. Vieira. 2016. Critérios para condução do teste de tetrazólio em sementes de araucária. Pesquisa Agropecuária Brasileira 51(1):61–68. doi: 10.1590/S0100-204X2016000100008

Ayala-Villegas, M.J., O.J. Ayala-Garay, V.H. Aguilar-Rincón, and T. Corona-Torres. 2014. Evolución de la calidad de semilla de *Capsicum annuum* L. durante su desarrollo en el fruto. Revista Fitotecnia Mexicana 37(1):79-87.

Belniaki, A. C., T.B. Michelon, E.S. Vieira, and M. Panobianco. 2020. Rapid results of peach palm seed viability: a methodological proposition for the tetrazolium test. Journal of Seed Science 42.

Bewley, J. D., and M. Black. 2013. Seeds: physiology of development and germination. Springer Science & Business Media.

Bánki, O., Y. Roskov, L. Vandepitte, R. DeWalt, D. Remsen, P. Schalk, T. Orrell, M. Keping, J. Miller, R. Aalbu, R. Adlard, E. Adriaenssens, C. Aedo, E. Aescht, N. Akkari, M. Alonso-Zarazaga, B. Alvarez, and F. Alvarez. 2022. Catalogue of Life Checklist. doi: 10.15468/RFFZ4X

Cano-Vázquez, A., M. López-Peralta, H.A. Zavaleta-Mancera, N. Cruz-Huerta, I. Ramírez-Ramírez, A. Gardea-Béjar, and V.A. González-Hernández. 2015. Variación en grados de latencia en semillas entre colectas de chile piquín (*Capsicum annuum* var. glabriusculum). Botanical Sciences 93(1):175-184. doi: 10.17129/botsci.138

Carrizo-García, C., M.H.J. Barfuss, E.M. Sehr, G.E. Barboza, R. Samuel, E.A. Moscone, and F. Ehrendorfer. 2016. Phylogenetic relationships, diversification and expansion of chili peppers (*Capsicum*, Solanaceae). Annals of Botany 118(1):35–51. doi: 10.1093/aob/mcw079

Copeland, L.O., and M.B. McDonald. 2001. Principles of Seed Science and Technology. 4th ed. Kluwer Academic Publishers, Boston, MA. doi: 10.1093/aob/mcf127

da Silva, F., L. Silva, E. Pereira, S. Barros, and F. Andrade. 2019. Initial development and tolerance of pepper species to salinity stress. Revista Caatinga 32: 826-833. doi: 10.1590/1983-21252019v32n327rc

De la Rosa, M., L. Arce, J. Villarreal, L. Ibarra, L, and J. Lozano. 2012. Germinación de semillas de chile simojovel (*Capsicum annuum* L.) previamente expuestas a NaCl y ácido giberélico. Phyton 81: 165–168

Dias, M.D.E, and S. Alves. 2008. Avaliação da viabilidade de sementes de *Panicum maximum* Jacq pelo teste de tetrazólio. Revista Brasileira de Sementes 30(3):152–158.

Eisvand, H.R., N. Fathi, and D. Goudarzi. 2015. Effects of some PGRs on seedling emergence and CAT and POD activity of maize under low temperature stress. Iranian Journal of Plant Physiology 5(3):1393-1402.

Elizalde, V., J.R. García, C.B. Peña-Valdivia, M.C. Ybarra, O.R. Leyva, and C. Trejo. 2017. Viabilidad y germinación de semillas de Hechtiaperotensis (Bromeliaceae). Revista de biología Tropical 65(1):153-165.

França-Neto, J.D.B., and F.C. Krzyzanowski. 2019. Tetrazolium: an important test for physiological seed quality evaluation. Journal of Seed Science 41:359-366. doi: 10.1590/2317-1545v41n3223104

González-Jara, P., A. Moreno-Letelier, A. Fraile, D. Piñero, and F. García-Arenal. 2011. Impact of human management on the genetic variation of wild pepper, *Capsicum annuum* var. *glabriusculum*. PLoS one 6(12): e28715

Hernández-Amasifuen, A.D., A.J. Pineda-Lázaro, and H.B. Díaz-Pillasca. 2019. Efecto de la luz y del ácido giberélico en la germinación *in vitro* de *Capsicum annuum* L. cv.'Papri King'. Biotecnología Vegetal 19(3):165-170

ISTA. 2022. International rules for seed testing. In International Seed Testing Association (Issue 1). doi:10.15258/istarules.2022.I

Jaiswal, V., V. Gahlaut, N. Kumar, and N. Ramchiary. 2021. Genetics, genomics and breeding of chili pepper *Capsicum frutescens* L. and other *Capsicum* species. Advances in Plant Breeding Strategies: Vegetable Crops 9:59-86

Kusumawardana, A., and B. Pujiasmanto. 2018. Tetrazolium test for evaluating viability of *Capsicum annuum* seeds. Nusantara Bioscience 10(3):142–145. doi: 10.13057/nusbiosci/n100302

Lakon, G. 1949. The topographical tetrazolium method for determining the germinating capacity of seeds. Plant Physiology 24(3):389-394.

Liu, Z., L. Kang, Y. Liu, J. Lv, X. Zou, Z. Liu, L. Ou, L. Kang, Y. Liu, J. Lv, G. Wei, B. Yang, S. Yang, W. Chen, X. Dai, X. Li, S. Zhou, Z. Zhang, Y. Ma, et al., Y. Zhang. 2017. Identification and characterization of novel microRNAs for fruit development and quality in hot pepper (*Capsicum annuum* L.). Gene 608:66–72. doi: 10.1016/j.gene.2017.01.020

Lizarde, N., E. Araiza, J. Guillermo, and M. Martínez. 2011. Evaluación de la germinación y crecimiento de Plántula de Chiltepín (*Capsicum annuum* L variedad *glabriusculum*) en invernadero. Revista Colombiana de Biotecnología 13(2):170–175

Martínez, J., T. Aquino-Bolaños, Y. Ortiz-Hernández, and S. Cruz-Izquierdo. 2019. Características de fruto y semilla de chile huacle (*Capsicum annuum* L.) producido en hidroponia. Idesia (Arica) 37(2):87–94. doi: 10.4067/S0718-34292019000200087

Mercado, S.A.S., and Y.M.O. Jaimes. 2022. Implementation of organic components to the culture medium to improve the *in vitro* propagation of *Cattleya warscewiczii* and *Cattleya gaskelliana*. South African Journal of Botany 148:352-359. doi: 10.1016/j.sajb.2022.05.002

Milošević, M., M. Vujaković, and D. Karagić. 2010. Vigour tests as indicators of seed viability. Genetika 42(1):103-118

Mireles-Rodríguez, E., N.L. Moctezuma-Balderas, S. Castro-Nava, R. Salazar-Hernández, H. Lucio-Castillo, and C. Pérez-Jasso. 2015. Preacondicionamiento en la germinación de cuatro colectas de chile piquín (*Capsicum annuum* var. *aviculare*) de Tamaulipas, México. Acta Agrícola y Pecuaria 1(3): 99-106

Moore, R.P. 1973. Tetrazolium staining for assessing seed quality. In: Seed Ecology. Ed. W. Heydecker. Butterworths, London. 347-353.

Mustafa, M., Y.F. Syahri, and M. Rauf. 2019. Selection of Chilli Pepper (*Capsicum frutescens* L.) for Salinity tolerance in seed germination. Agrotech Journal 4(2):83-90.

Oh, W., and K.S. Kim. 2014. Light intensity and temperature regulate petiole elongation by controlling the content of and sensitivity to gibberellin in *Cyclamen persicum*. Horticulture, Environment, and Biotechnology 55(3):175-182

Ozbay, N. 2018. Studies on seed priming in pepper (*Capsicum annuum* L.). In Advances in seed priming. Springer, Singapore. 209-239. doi: 10.1007/978-981-13-0032-5_12

Pickersgill, B. 2016. Chile peppers (*Capsicum* spp.). In Ethnobotany of Mexico. 417-437. Springer, New York, NY.

Pico-Mendoza, J., M. Pinoargote, L. Madrid, J. Flor, J. Álava, G. Sancán. et al., K. Quiroz. 2020. Germination capacity of Annona deceptrix (Westra) H. Rainer (Annonaceae) an endemic and endangered species in Manabí, Ecuador. Indian Journal of Agricultural Research 54(3):329-335.

Ramchiary, N., and C. Kole. 2019. The *Capsicum* Genome. In N. Ramchiary & C. Kole (Eds.), *Springer Nature Switzerland AG* (primera). Springer Nature Switzerland AG. https://link.springer.com/content/pdf/10.1007%2F978-3-319-66117-9.pdf

Rami, K. and A. Patel. 2014. Effect of seed size on seed germination and seedling vigour in chilli (*Capsicum annuum* L.). International Journal of Agriculture and Food Science Technology 5(8):871-876

Ravindran, P., and P.P. Kumar. 2019. Regulation of seed germination: The involvement of multiple forces exerted via gibberellic acid signaling. Molecular plant 12(1):24-26.

Salazar-Mercado, S., and E. Botello-Delgado. 2018. Viabilidad de semillas de *Glycine max* (L.) utilizando la prueba de tetrazolio. Revista de Investigación Agraria y Ambiental 9(2):91–98. doi: 10.22490/21456453.2270

Salazar-Mercado, S., J. Quintero-Caleño, and L. Moreno-Rozo. 2020. Improvement of the methodology of the tetrazolium test using different pretreatments in seeds of the genus Epidendrum (orchidaceae). Journal of Seed Science 42. doi: 10.1590/2317-1545v42231028

Saldivar-Iglesias, P., A. Laguna-Cerda, F. Gutiérrez-Rodríguez, and M. Dominguez-Galindo. 2010. Ácido giberélico en la germinación de semillas de Jaltomata procumbens (CAV.) J.L. Gentry. Agronomía Mesoamericana 21(2):327–331

Sandoval-Rangel, A., A. Tapia González, M. Cabrera- De la Fuente, J.A González Fuentes, and A. Benavides-Mendoza. 2018. Edad, beneficio y ácido giberélico afectan la germinación y producción de planta de chile piquín. Revista Mexicana de Ciencias Agrícolas 20:4199–4209. doi: 10.29312/remexca.v0i20.990

Siri, B., K. Vichitphan, P. Kaewnaree, S. Vichitphan, and P. Klanrit. 2013. Improvement of quality, membrane integrity and antioxidant systems in sweet pepper (*'Capsicum annuum'* Linn.) seeds affected by osmopriming. Australian Journal of Crop Science 7(13):2068-2073.

Stockert, J.C., R.W. Horobin, L.L. Colombo, and A. Blázquez-Castro. 2018. Tetrazolium salts and formazan products in Cell Biology: Viability assessment, fluorescence imaging, and labeling perspectives. Acta histochemica 120(3):159-167

Srivastava, A.K., J. Suresh Kumar, and P. Suprasanna. 2021. Seed 'primeomics': plants memorize their germination under stress. Biological Reviews 96(5):1723-1743

Tombegavani, S.S., B. Zahedi, S. Mousavi Fard, and A. Ahmadpour. 2020. Response of germination and seedling growth of pepper cultivars to seed priming by plant growth regulators. International Journal of Horticultural Science and Technology 7(1):59-68. doi: 10.22059/ijhst.2020.274293.275

Torres, R.C., and K.C.A. de Souza Borges. 2013. Ação da giberelina no crescimento de pimenta (*Capsicum frutescens*). Cadernos UniFOA 8(1):11-16

Tripathi, D.K., S.R. Yadav, K. Mochida, and L.S.P. Tran. 2022. Plant growth regulators: true managers of plant life. Plant and Cell Physiology 63(12):1757-1760

Tripodi, P., and S. Kumar. 2019. The Capsicum crop: an introduction. In The *Capsicum* Genome (pp. 1-8). Springer, Cham.Vichiato-Medeiros, M., Vichiato, M., de Castro, D., Ferreira, L., & Pasqual, M. (2007). Alongamento de plantas de Dendrobium nobile Lindl. com pulverização de ácido giberélico. Ciência e Agrotecnologia 31(1):16–20

Yamamoto, S., and E. Nawata. 2006. Germination characteristics of *Capsicum frutescens* L. on the Ryukyu islands and domestication stages of *C. frutescens* L. in Southeast Asia. Japanese Journal of Tropical Agriculture 50(3):142-153. doi: 10.11248/jsta1957.50.142