

## INFLUENCE OF GIBBERELIC ACID AND DIFFERENT NaCl CONCENTRATIONS ON SEED GERMINATION OF WILD PIQUIN PEPPER (*CAPSICUM ANNUUM* L. VAR. *GLABRIUSCULUM*) COLLECTED IN THE HUASTECA VERACRUZANA

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**Abstract.** Piquin pepper (*Capsicum annuum* L. var. *glabriusculum*) is a wild chili primarily harvested for community uses. Like most wild species, they have a low germination rate, mainly due to long dormancy periods, which are an obstacle to domestication and commercial production. Germination depends on factors such as light, phytohormones, temperature, and humidity, among others. Plant hormones play a key role in maintaining dormancy and promoting seed germination; gibberellic acid (GA<sub>3</sub>) helps break seed dormancy. This study aimed to evaluate the effect of seed priming with GA<sub>3</sub> or the combined NaCl-GA<sub>3</sub> treatment to improve the germination rate of Piquin pepper seeds from the Huasteca region in Veracruz, Mexico. Seeds were treated with different concentrations and combinations of NaCl and GA<sub>3</sub>. In the first approach, seeds were treated individually with 100, 250, 350, and 450 mg L<sup>-1</sup> of GA<sub>3</sub>, as well as 100, 250, 350 and 450 mM of NaCl, plus one control without treatment. The treatment of 250 mg L<sup>-1</sup> of GA<sub>3</sub> reached 80% germination on day 12. In a second approach, we tested the effect of 250 mg L<sup>-1</sup> of GA<sub>3</sub> alone, 500 mM of NaCl alone, the combination of 500 mM of NaCl with 250 mg L<sup>-1</sup> of GA<sub>3</sub> and a control without treatment. The 250 mg L<sup>-1</sup> of GA<sub>3</sub> treatment reached 100% of germination, while the 500 mM NaCl treatment combined with 250 mg L<sup>-1</sup> of GA<sub>3</sub> reduced seed germination to 60% after 24 days. In the third approach, 100 mM of NaCl alone, 350 mM of NaCl alone, 250 mg L<sup>-1</sup> of GA<sub>3</sub> alone, a combination 100 mM of NaCl with 250 mg L<sup>-1</sup> of GA<sub>3</sub>, a combination 350 mM of NaCl with 250 mg L<sup>-1</sup> of GA<sub>3</sub>, and control without treatment were evaluated. In this approach, treatment with 250 mg L<sup>-1</sup> of GA<sub>3</sub> significantly increased radicle length compared with the other conditions. Our results showed that only a gibberellic acid treatment could improve the Piquin pepper wild seed germination by over 80%, providing a low-cost alternative for germination seeds of wild species, while also aiding in the preservation of this genetic and food resource.

**Keywords:** *Piquin pepper, germination, seed priming*

### Introduction

Chili Piquin, or Piquin pepper (*Capsicum annuum* L. var. *glabriusculum* (Dunal) Heiser and Pickersgill), is distributed from the southern United States to the low altitude

regions of Peru and Mexico. It is located mainly in tropical deciduous forests, along roadsides, in orchards, pastures, and under remnant vegetation on the banks of cultivated lands. Piquin pepper is considered the ancestor of all types of chili peppers currently known (Hayano et al., 2016; Guadalupe et al., 2019; Cano et al., 2021; Cervantes et al., 2022). Wild plants of Piquin pepper are a valuable genetic resource for diversity, as well as drought and cold tolerance. However, they are currently under strong pressure due to the form and characteristics of fruit collection, giving rise to an irrational exploitation practice that have caused the disappearance of the species in some regions, particularly those near population centers (Hayano et al., 2016; Mares and Valiente, 2019). Piquin fruit is highly valued and appreciated, often reaching a price several times higher than other varieties, such as Serrano and Jalapeño. During the high supply season, it replaces other chili varieties due to its pleasant flavor and degree of pungency. Additionally, it is considered less irritating to the digestive system compared to the other peppers (Villalón et al., 2014; Paredes et al., 2019; Barboza et al., 2022).

Piquin pepper is cultivated mainly as wild type, and due to environmental adaptation, there is a marked heterogeneity in the size, color, and form of the fruit (Valiente and Gutierrez, 2016; Díaz-Sánchez et al., 2021). Unfortunately, despite its commercial importance, the domestication of the Piquin pepper has not been fully achieved due to problems related to low and erratic seed germination, morphological and genetic variability, and limited knowledge of its environmental physiology (Rodríguez-Uribe et al., 2014; Cano-Vázquez et al., 2015; Jiménez et al., 2022). The seeds of the wild chili plants have a latency period after fruit maturation, which prevents immediate germination but ensures the survival of the species (Urbanova and Leubner, 2016). In chili Piquin, the seed germination rate is low and varies significantly depending on the variety and natural environment. This low germination rate is attributed to physical or physiological dormancy, which is caused by low permeability and hardness of the seed coat, as well as the strong dormancy of the embryo (Rodríguez et al., 2014; Corozo Quiñónez et al., 2023). A low germination seed rate is generally an undesirable characteristic in crops, where rapid germination and growth are required. Different techniques have been reported to solve this problem, including chemical and physical scarification, conditioning, or seed treatment prior to sowing, such as with gibberellic acid (GA<sub>3</sub>), potassium nitrate, and hydrogen peroxide, among others (De la Rosa et al., 2012; Cano-Vázquez et al., 2015; Jiménez et al., 2022).

Various treatments have been used to break seed dormancy. It determines the capacity to use the seeds that are available, in the case of old seeds, the possibility of recovering them. The seeds of wild plants represent an important reservoir as many of them show greater tolerance to stressful environmental conditions. However, germination efficiency is always a limiting factor, especially in the case of old seeds. Some of the methods used are thermal shock with cold or hot water treatment, use of some treatments involving chemical or hormonal agents. In the case of old wild seeds, the best treatments have been with hormones, particularly gibberellins (Nautiyal et al., 2023).

The plant hormone GA<sub>3</sub> can break seed dormancy and promote germination (Bentsink and Koornneef, 2008; Bassel, 2016; Beltrán-Burboa, et al., 2020). Pre-conditioning treatment seeds with GA<sub>3</sub> contribute to interrupting the dormancy and increasing the germination percentage of chili under optimal conditions (Araiza et al., 2011; Cano-Vázquez et al., 2015). In crops, the germination and seedling development stages are mainly affected by biotic and abiotic stresses, such as high soil salinity and

environmental factors like temperature and light. Soil salinity can affect germination due to its osmotic and ionic components, shown by decreased germination rate and prolonged germination time. However, at suitable concentrations of NaCl, seed priming can enhance seed germination and seedling growth, and in some cases, it has even been shown to improve tolerance to salinity stress conditions (Bojović et al., 2010; Elouaer and Hannachi, 2012).

Hernández-Verdugo et al. (2010) obtained 45.3% germination by placing seeds in Petri dishes with agar enriched with 500 ppm GA<sub>3</sub> for 10 h of light. Osmotic conditioning with polyethylene glycol, GA<sub>3</sub>, and potassium nitrate is an alternative method to increase the seed germination and seedling emergence of *Capsicum annuum* Linnaeus cv. “Chile Mulato” also known as “Chile Ancho” (Cortez-Baheza et al., 2011). De la Rosa et al. (2012) reported that Simojovel pepper seeds, a native landrace of Piquin pepper from Chiapas State, southern Mexico, preconditioned with 0.5 M NaCl and then treated with GA<sub>3</sub> (350 mg L<sup>-1</sup>), significantly increased their germination percentage (91.7%). Few studies have been performed on Piquin pepper germination, but all show conflicting results, indicating that germination is heavily dependent on the origin of seed accessions and their genetic diversity (Rodríguez-Uribe et al., 2014; Cano-Vázquez et al., 2015). This study aimed to evaluate the effect of seed priming with GA<sub>3</sub>, NaCl, and a combined NaCl-GA<sub>3</sub> treatment to improve the germination rate of wild Piquin pepper seeds from Huasteca Veracruzana. This information will be useful for the development of seedling production programs. The interest in the domestication of Piquin pepper is important, and then producers must guarantee high seed germination percentages and the conservation of this genetic resource.

## Materials and methods

### *Plant material*

Piquin pepper seeds were collected from the wild population in San Juan Otonopec (21°12'38.1"N; 97°55'10.8"S), Veracruz, México, during October and November of 2015. The seeds from three generations of self-crossing were selected to reduce the genetic variability and produce homogeneous lines. During 2017, fresh seeds were collected and submerged in water to separate by flotation, disinfected in 1% sodium hypochlorite (NaClO, Cloralex<sup>®</sup>) for 15 min and then washed with distilled water.

### *Seed germination assay*

In the first approach, four concentrations of gibberellic acid (GA<sub>3</sub>): 100, 250, 350 and 450 mg L<sup>-1</sup> and four concentrations of NaCl: 100, 250, 350 and 450 mM were evaluated. A control treatment, in which the seeds were irrigated with distilled water, was also included. The treatment with the highest germination percentage was selected for the following evaluations. To evaluate germination, 20 uniform seeds were placed in each replicate of each treatment and grown with the treatment solutions in Petri dishes (9 cm in diameter). Germination was assessed at 12, 18, and 24 days after sowing. The Petri dishes were incubated in a growth chamber (Lab-Line Instruments) with a 12 h light/12 h darkness cycle, at 23 ± 1°C and 70% relative humidity (RH).

In a second approach, a preconditioning treatment with NaCl (priming) was performed in combination with the best GA<sub>3</sub> concentration previously selected. This experiment included the following conditions: (1) seeds were germinated in distilled

water as a control condition; (2) seeds were soaked for 24 h in water, dried for 6 h at 37°C, and then treated with 250 mg L<sup>-1</sup> of GA<sub>3</sub>; (3) seeds were soaked for 24 h in 500 mM of NaCl, dried for 6 h at 37°C, and treated with 250 mg L<sup>-1</sup> of GA<sub>3</sub>; and (4) seeds were soaked for 24 h in 500 mM of NaCl, dried for 6 h at 37°C. We included these last two treatments because it was reported that a native landrace of Piquin pepper from Chiapas State (Mexico), preconditioned with 500 mM NaCl and then treated with GA<sub>3</sub>, significantly increased its germination percentage to 91.7% (De la Rosa et al., 2012). For each replicate in each treatment, 20 uniform seeds were placed in Petri dishes (9 cm in diameter) with the treatment solutions. A double layer of sterile filter paper was placed in each Petri dish, and 7 mL of the respective treatment solution, either GA<sub>3</sub> (Raligeb<sup>®</sup>) or distilled water, was added. The Petri dishes were incubated in a growth chamber (Lab-Line Instruments) with 12 h light/12 h darkness cycle, at 23 ± 1°C and 70% RH. The number of germinated seeds was recorded at 12, 18, and 24 days after sowing. Seeds were considered germinated when the radicle length reached 0.2 mm. The total germination percentage is an estimate of the seed viability.

In the third approach, root length was measured in chili seedlings. The root length of 20 chili seedlings per treatment was evaluated and measured 18 and 24 days after sowing. This approach included the following treatments: (1) seeds were germinated in distilled water as a control condition; (2) seeds were soaked for 24 h in water, dried for 6 h at 37°C, and then treated with 250 mg L<sup>-1</sup> of GA<sub>3</sub>; (3) seeds were soaked for 24 h in 100 mM of NaCl, dried for 6 h at 37°C, and treated with 250 mg L<sup>-1</sup> of GA<sub>3</sub>; (4) seeds were soaked for 24 h in 100 mM of NaCl, and dried for 6 h at 37°C; (5) seeds were soaked for 24 h in 350 mM of NaCl, dried for 6 h at 37°C, and treated with 250 mg L<sup>-1</sup> of GA<sub>3</sub>; (6) seeds were soaked for 24 h in 350 mM of NaCl, and dried for 6 h at 37°C.

### **Statistical analysis**

A completely randomized design with three repetitions was used in both the first and second approaches. A completely randomized design with twenty repetitions was used in the third approach. Statistical evaluations were performed using analysis of variance (ANOVA) with the statistical program SAS (SAS Institute, 2003). For the comparison of means, the Tukey test ( $p \leq 0.05$ ) was applied. Germination values, expressed as percentages, were transformed using the arc sine function  $\sqrt{X/100}$ . Graphs were constructed using Microsoft Excel 2007<sup>®</sup> (Microsoft Corp., USA).

### **Results and discussion**

To improve the germination rate of wild Piquin pepper seeds, treatments with GA<sub>3</sub> and a combined NaCl-GA<sub>3</sub> treatment were applied. In the first approach, the germination of Piquin pepper seeds was evaluated in four concentrations of GA<sub>3</sub> (100, 250, 350, and 450 mg L<sup>-1</sup>) and four concentrations of NaCl (100, 250, 350, and 450 mM). The analysis of variance results in the first stage showed highly significant differences among the treatments  $p \leq 0.01$  (Table 1). We observed that under 100 mg L<sup>-1</sup> GA<sub>3</sub>, the germination rate was around 35% at 24 days, which was lower than control, although the difference was not significant (Fig. 1). Under 250 L<sup>-1</sup> GA<sub>3</sub>, the germination rate was significantly increased to 80% within the first 12 days after planting. Under 350 mg L<sup>-1</sup> GA<sub>3</sub>, the germination rate was increasing over time and reached around 70% at 24 days after planting. Under 450 mg L<sup>-1</sup> GA<sub>3</sub>, the germination rate was around 65%, slightly higher than the control. Under 100 mM NaCl, the germination rate was below

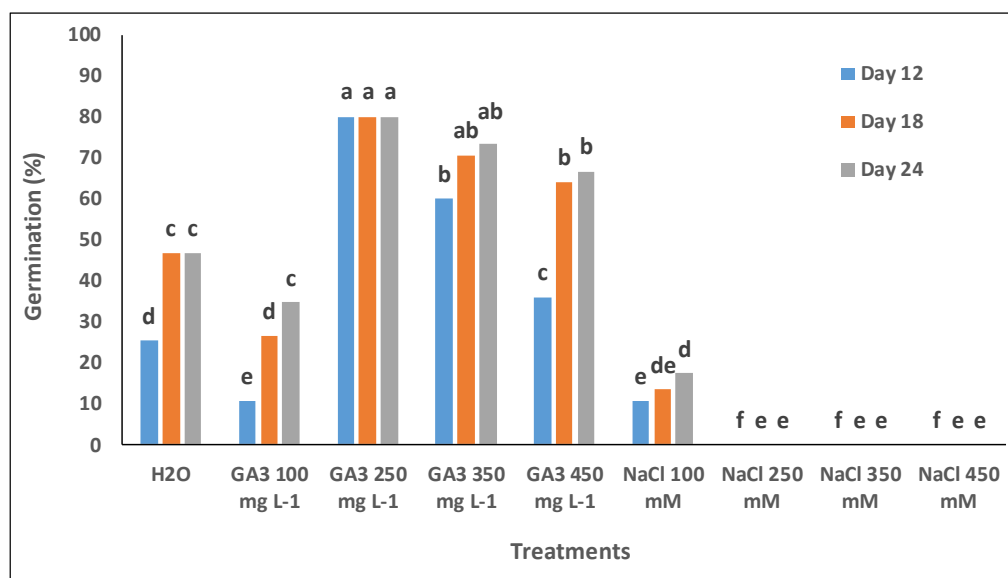
20%, significantly lower than the control. No seed germinated under NaCl with concentrations above 250 mM. These results showed that this germplasm is sensitive to pre-treatment with NaCl at concentration as low as 100 mM, while treatments with 250 mg L<sup>-1</sup> GA<sub>3</sub> alone significantly promote an increase in the germination rate of Piquin pepper.

**Table 1.** Mean squares and statistical significance of the evaluation of germination of Piquin pepper seeds by applying treatments with gibberellic acid (GA<sub>3</sub>) to promote germination

Sources of variation	Degrees of freedom	Germination on day 12 (%)	Germination on day 18 (%)	Germination on day 24 (%)
Treatments	8	1594.8 **	2005.9 **	2061.6 **
Error	18	2.5	9.1	7.0
C.V. (%)		6.6	10.0	8.4
Mean		24.7	33.4	35.4

CV = Variation coefficient. \*\*, \* = Highly significant and significant with  $p \leq 0.01$  and  $p \leq 0.05$ , respectively; ns = not significant

Saline conditions have been used as seed priming treatments in various crops. Seed priming of Coahuila Piquin pepper with 500 mM of NaCl and 350 mg L<sup>-1</sup> of GA<sub>3</sub> increased the germination from 24.75 to 91.75% (De la Rosa et al., 2012). Our results indicate that the germination rate of Piquin pepper seeds from Veracruz was significantly reduced at a concentration to low as 100 mM of NaCl (Fig. 1).

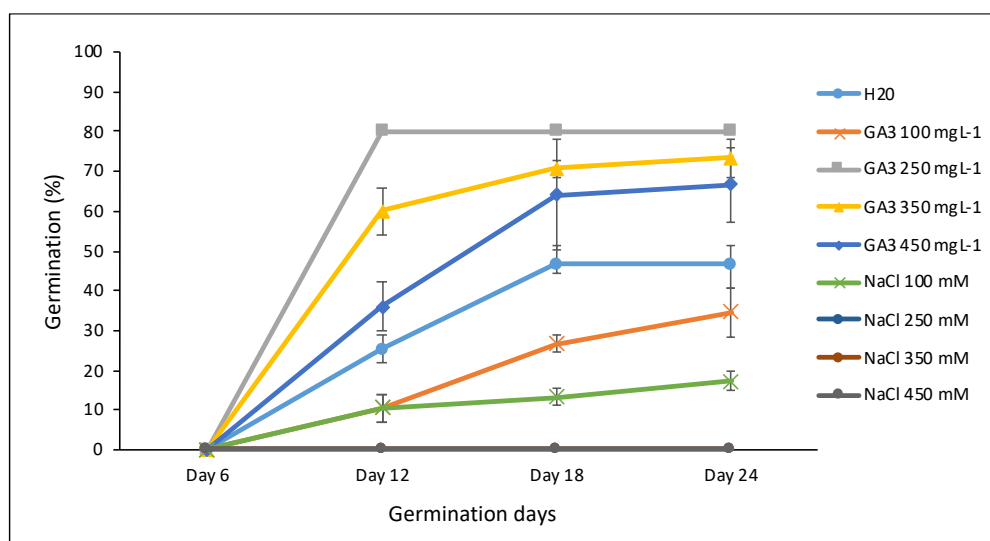


**Figure 1.** Seed germination percentage of Piquin pepper with GA<sub>3</sub> and NaCl treatments. Means with different letter are statistically different (Tukey, 0.05)

Plant hormones such as gibberellic acid regulate the seed germination through the induction of the synthesis of  $\alpha$ -amylase in the aleurone layer. This enzyme is secreted into the starchy endosperm, where it facilitates hydrolysis, mobilizes reserves, and stimulates germination. The exogenous gibberellic acid increases germination and

seedling growth by enhancing the availability of endogenous gibberellic acid (Gupta and Chakrabarty, 2013).

The kinetics of the germination behavior (Fig. 2) generally shows that the treatment with 250 mg L<sup>-1</sup> of GA<sub>3</sub> resulted in the most significant response in the germination, being more noticeable from the 6<sup>th</sup> to 12<sup>th</sup> day. However, when the GA<sub>3</sub> concentration was increased to 450 mg L<sup>-1</sup>, the germination rate was reduced, but it was slightly higher level than that of the control. These results suggest that the application of a low concentration of GA<sub>3</sub> has positive effect on gemination, while a higher concentration of GA<sub>3</sub> may have opposite effects. Previous reports have also shown that higher concentrations of gibberellins can negatively affect both germination and yield (Pichardo-González et al., 2018; Zhu et al., 2019). This effect may be due to imbalances in endogenous gradients with other hormones (Shuai et al., 2017; Vishal and Kumar, 2018).



**Figure 2.** Kinetics of germinative behavior of Piquin pepper seeds with GA<sub>3</sub> and NaCl treatments. Vertical bars correspond to standard error

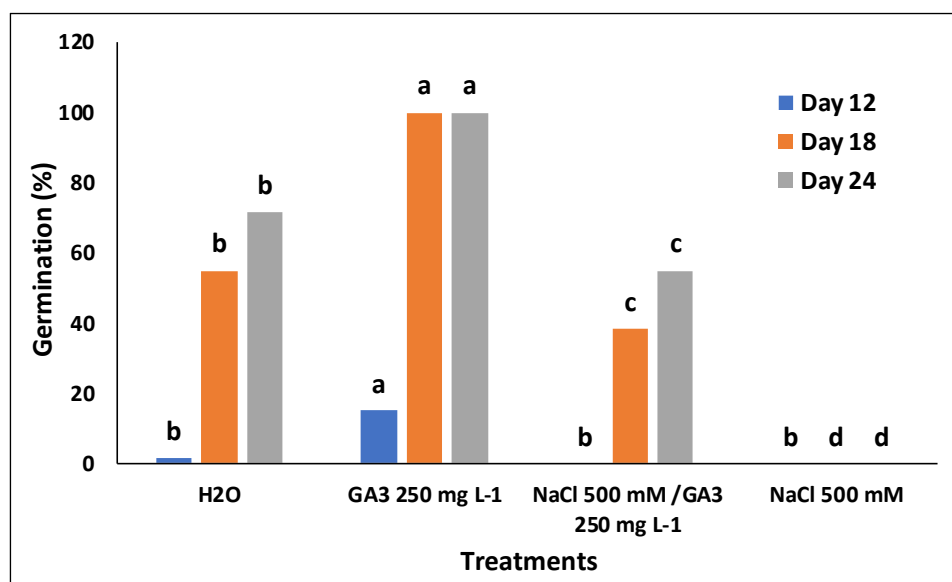
In the second approach, seeds were preconditioned with NaCl 100 mM and exposed to 250 mg L<sup>-1</sup> GA<sub>3</sub>. The ANOVA analysis showed that the germination treatments evaluated presented highly significant differences ( $p \leq 0.01$ ) in the three periods evaluated (Table 2). On the 12<sup>th</sup> day, the 250 mg L<sup>-1</sup> of GA<sub>3</sub> treatment achieved 100% germination (Fig. 3). The combined treatment of 500 mM of NaCl with 250 mg L<sup>-1</sup> of GA<sub>3</sub> significantly reduced the germination rate to 60% compared to GA<sub>3</sub> alone (Fig. 3). In the presence of only 500 mM of NaCl, no seeds germinated. This result allows suggest that 500 mM of NaCl has a negative effect on germination, and that a GA<sub>3</sub> treatment alone is sufficient to significantly improve germination in Piquin pepper wild seeds.

The results showed that by day 12, the H<sub>2</sub>O + GA<sub>3</sub> treatment had the highest and statistically significant germination percentage, around 15%, which reached 100% by day 18. The NaCl + GA<sub>3</sub> treatment had a germination percentage of around 38% until day 18, which was lower than the control treatment (Fig. 3). These results suggest that during the first days, the GA<sub>3</sub> treatment had the most pronounced positive effect on the germination of Piquin pepper wild seed.

**Table 2.** Mean squares and statistical significance of the evaluation of germination of piquin chili seeds by applying treatments with gibberellic acid (GA<sub>3</sub>) and sodium chloride (NaCl)

Sources of variation	Degrees of freedom	Germination on day 12 (%)	Germination on day 18 (%)	Germination on day 24 (%)
Treatments	3	354.2 **	4100.1 **	4162.6 **
Error	8	13.9	2.8	5.3
C.V. (%)		55.0	3.8	4.7
Mean		4.1	48.3	56.6

CV = Variation coefficient. \*\*, \* = Highly significant and Significant with  $p \leq 0.01$  and  $p \leq 0.05$ , respectively; ns = not significant



**Figure 3.** Combined effects of NaCl and GA<sub>3</sub> treatment on germination rate of Piquin pepper wild seeds. Means with different letter are statistically different (Tukey, 0.05)

In the third approach, root length was measured in Piquin pepper seedlings. The ANOVA showed that the treatments evaluated had highly significant differences ( $p \leq 0.01$ ) in root length measurement at 18 and 24 days after sowing (Table 3). Under 250 mg L<sup>-1</sup> of GA<sub>3</sub>, radicle length was significantly increased around of 230% on day 24, compared with the control condition (Fig. 4). This positive effect was inhibited when 250 mg L<sup>-1</sup> of GA<sub>3</sub> was combined with 100 mM of NaCl in the pre-conditioning treatment, obtaining radicle length values like those of the control. Radicle length was inhibited at NaCl concentrations above 100 mM (Fig. 4). These results indicate that Piquin pepper radicle length is significantly regulated by GAs.

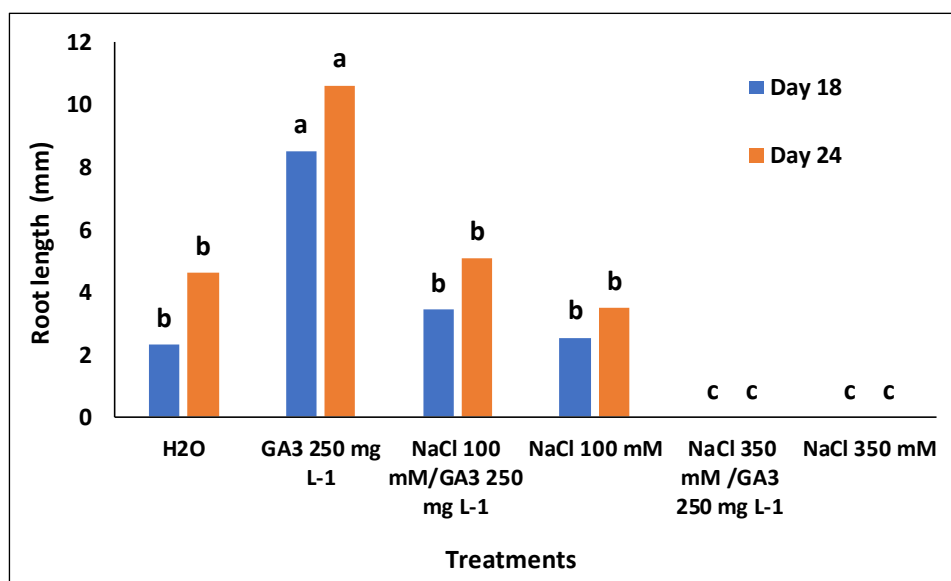
Gibberellic acid has proved to be a stimulant that rapidly increases germination percentage, likely because this, hormone treatment reduces the resistance imposed by the compounds present in the seed endosperm to allow seedling development (Richards et al., 2001; Cano-Vazquez et al., 2015). On the other hand, Petruzzelli et al. (2003) and Nezar et al. (2020) associated the accumulation of class I,  $\beta$ -1,3-glucanase and chitinase in response to different phytohormones such GA<sub>3</sub>, suggesting that  $\beta$ -1,3-glucanase contributes to the hydrolysis of cell wall components, resulting in endosperm

weakening at the site of radicle protrusion (Wu and Bradford, 2003). Additionally, Wu et al. (2001) showed that the genes encoding chitinase (*Chi9*) and  $\beta$ -1,3-glucanase (*GluB*) are expressed during seed germination in tobacco, chili, and tomato.

**Table 3.** Mean squares and statistical significance of the evaluation of root length in seedlings by applying treatments with gibberellic acid (GA3) and sodium chloride (NaCl) in Piquin pepper seeds

Sources of variation	Degrees of freedom	Root length in seedlings on day 18 (mm)	Root length in seedlings on day 24 (mm)
Treatments	5	195.4 **	309.4 **
Error	114	4.3	4.2
C.V. (%)		74.2	51.8
Mean		2.7	3.9

CV = Variation coefficient. \*\*, \* = Highly significant and Significant with  $p \leq 0.01$  and  $p \leq 0.05$ , respectively; ns = no significant



**Figure 4.** Effects of NaCl and GA<sub>3</sub> treatments on radicle length in Piquin pepper seedlings of wild seeds. Means with different letter are statistically different (Tukey, 0.05)

Few studies have been performed in Piquin pepper germination, however, all of them show contrasting results (Cano-Vázquez et al., 2015; Hernández-Verdugo et al., 2010; De la Rosa et al. 2012; Quintero et al., 2018). On one hand, a positive effect has been reported using both high (5000 mg L<sup>-1</sup>) and low doses (200 mg L<sup>-1</sup>) of GA<sub>3</sub>. Garcia-Federico et al. (2010) reported an increase in the germination rate from 33% to 82% in Piquin pepper seeds from Queretaro state (Mexico), treated with 500 ppm GA<sub>3</sub>. De la Rosa et al. (2012) evaluated seeds of Simojovel pepper (a native landrace of Piquin pepper from Chiapas State, Mexico) treated with GA<sub>3</sub> which showed 25% germination in control treatment, while with 400 mg L<sup>-1</sup> GA<sub>3</sub> the germination rate increased to 78%. Cano-Vazquez et al. (2015) used pre-conditioned seeds of Piquin pepper from 16 accessions across 7 states in Mexico and USA with GA<sub>3</sub> at 5000 ppm, obtaining an

average germination rate of 59% in 14 out of 16 accessions. On the other hand, Quintero et al. (2018) reported that GA<sub>3</sub> at low doses, such as 200 mg L<sup>-1</sup>, is necessary to increase the germination rate in Piquin pepper seeds from Tamaulipas and San Luis Potosi states. The seed age is another factor that influences the germination rate for Piquin pepper seeds. The highest germination percentages of wild Piquin peppers seeds from Coahuila, Mexico, were obtained using GA<sub>3</sub> at 5000 mg L<sup>-1</sup> in the seed that were 2 and 4 months old (Sandoval-Rangel et al., 2018). These conflicting results suggest that the germination rate in Piquin pepper is a trait highly dependent on the origin of seed accessions and their genetic diversity (Oyama et al., 2006; Hernandez-Verdugo et al., 2012; Cano-Vasquez et al., 2015; Quintero et al., 2018). In this sense, there is no universal protocol to promote germination in Piquin pepper. In this study, a concentration of 250 mg L<sup>-1</sup> of GA<sub>3</sub> improved both the germination rate and the radicle length in wild Piquin pepper seeds collected from the Huasteca Veracruzana.

## Conclusions

In this study, we demonstrated that the optimal concentration for enhancing germination over 80% in wild Piquin pepper seeds collected from the Huasteca Veracruzana is 250 mg L<sup>-1</sup> of gibberellic acid, applied in the form of Raligeb®. One of the advantages of our strategy for improving wild seeds germination is the use of low-cost, commercially available GA<sub>3</sub>-based compounds, which will allow us to preserve this genetic and food resource.

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