Relieving dormancy and improving germination of Piquín chili pepper (Capsicum annuum var. glabriusculum) by priming techniques

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Abstract: The effects of different priming techniques were evaluated to improve the dormancy and germination of wild seeds of “Piquín” chili pepper. Three experiments were designed for presowing treatment of seeds: a) chemical seeds digestion; b) halopriming (with K⁺ or NH₄⁺ of NO₃, SO₄²⁻ or Cl⁻) at different priming times (24, 48 or 72 h) and osmotic potential (-5, -10 or -15 atm) and c) previously selected halopriming (KNO₃ and NH₄NO₃) + Gibberellic acid (GA₃, at 100 or 200 ppm) were tested. Digestion treatments did show a negative effect on seed germination. Recommended values of osmotic potential (Ψₛ), to improve Piquín chili seed germination, must be between -10 and -15 atm (-1.0 and -1.5 MPa) and the priming time must be between 48 and 72 hours. Priming techniques can considerably reduce Capsaicinoids content on seeds, improve dormancy, seed germination performance, and increase the rate and uniformity of seedling establishment. KNO₃ and secondly GA₃ treatments may improve rapid and uniform germination and seedling emergence. The results provide basic information to develop guidelines for commercial establishment of Piquín pepper crops.

Keywords: Wild chili pepper; domestication; seed germination; capsaicinoids content; halopriming; gibberellic acid.

1. Introduction

Chili “Piquín” or “chiltepín”, [Capsicum annuum var. glabriusculum (Dunal) Heiser & Pickersgill); syn. C. annuum var. aviculare (Dierb.) D’Arcy & Eshbaugh], is distributed from Colombia, Central America, and Mexico to the southwestern United States. The natural populations of “chiltepín” are considered an important genetic resource for pepper crop improvement [1]. This species is of great significance in the culture and identity of indigenous peoples of Mexico who usually harvested its fruits of wild plants [2]. The heat of chili pepper is due to the accumulation of capsaicinoids, a group of related alkaloids unique to Capsicum. Capsaicinoids are produced in the fruit placenta and transferred to the seeds during fruit maturation [3]. In highland regions where it occurs, is an important part of the...
local economy, especially in the time of harvest, generating employment and income for rural communities. This activity might threaten the genetic diversity in this species, affecting habitat degradation of natural populations of wild pepper [4]. This problem could be solved by limiting the collection of wild populations and increasing their cultivation as a crop, in turn generating economic resources derived from this activity [5]. While there is basic information that allows for developing guidelines for its cultivation [6], more research, related to germination, stand establishment and crop development and productivity, is necessary to develop commercial Piquín pepper crops.

Domestication of Piquín pepper plants have not been fully developed because problems are encountered related to low and erratic seed germination, morphologic and genetic variability, and limited environmental physiology information [6–8]. Some authors suggest that germination of its seeds is restricted by physiological dormancy [9] and is achieved after passing through the digestive tract of certain birds [10]. Seeds of many species remain viable after passing through the digestive tracts of animals, with varying effects on germination [11]. Seed dormancy is generally an undesirable characteristic in agricultural crops, where rapid germination and growth are required. Extensive domestication and breeding of crop species have ostensibly removed most dormancy mechanisms present in the seeds of their wild ancestors. Studies have reported a myriad of methods to break seed dormancy, including chemical, mechanical, thermal, and hormonal seed treatments [12,13].

The beneficial effects of priming on the vigor, germination of seeds and establishment of the seedlings is known since the times of Pliny the elder (A.D. 23-79) [14]. Seed priming is a presowing treatment involves the controlled hydration of seeds, sufficient to allow pregerminative metabolic events to take place but insufficient to allow primary root protrusion through the seed coat [13]. It also involves complex physiological and biochemical process which offers an effective means to improve seed quality [15], seed germination and vigor [16]. Priming treatments are widely applied by seed companies to increase the germination rate and uniformity of seedling establishment of commercial vegetable and flower seeds [17]. Primed seeds are equipped with advanced germination and exhibit improved germination rate and uniformity [18]. The benefits, associated with certain physiological, biochemical, cellular and molecular changes [17], include rapid, uniform and increased germination, improved seedling vigor and growth under a broad range of environments resulting in better stand establishment [19]. Different priming treatments such as hydrominering (soaking in water), halopriming (soaking in inorganic salt solutions), osmopriming (soaking in solutions of different organic osmotic molecules), thermopriming (treatment of seed with low or high temperatures) or solid matrix priming (treatment of seed with solid matrices) can be effectively employed to prime a large number of hot pepper seeds at one time [13,20]. Halopriming can affect osmoregulation in seeds by the active uptake of inorganic ions, promoting K⁺ and Ca²⁺ absorption and decreasing Na⁺ and Cl⁻ accumulation. Potassium plays an important role in balancing membrane potential and turgor, activating enzymes, and regulating osmotic pressure in cells [17]. Some authors hypothesized that capsaicinoids could have some allelopathic effect on pepper seed germination [3]. Capsaicinoids are a well-established allelochemical and has been shown to reduce root and shoot growth or suppress germination in several plant species [21]. The effects of incorporating plant growth regulators into the priming solution have also been indicated to improve the germination and the growth of pepper seedlings [22,23], and other vegetables [24,25].

The objective of this study was to evaluate both the response rate of wild seeds of Chili Piquín (Capsicum annuum var. glabriusculum) to break dormancy and improve germination rate through seed
priming and halopriming integrated with gibberellic acid (GA₃) treatments. This information is needed to help in the development of sound and reliable guidelines for seedling production of Piquín pepper and contribute to its domestication.

2. Materials and Methods

2.1. Plant materials:
Fruit of Chili Piquín were collected from different wild population in the States of Tamaulipas and San Luis Potosí, in Northeastern Mexico. Seed extraction was carried out manually, macerating fruits of each wild population and dipping them in water to separate the pure seed from impurities. Seeds from different wild population were disinfected, as separate seed lot, in 1% sodium hypochlorite solution for 15 min. to eliminate seed borne microorganisms [26,27]. After disinfection, seeds were dried on shade for two weeks, then stored for two more weeks, at laboratory hot and dry conditions, before giving different treatments.

2.2. Seed treatments:
To achieve the proposed objective, a series of three consecutive experiments were designed for pre-sowing treatment of seeds. Following every treatment all seeds were rinsed under running tap water for 3 minutes and then with distilled deionized water (ddH₂O) for 1 min. After rinsing, seeds were surface dried by placing them between paper towels for 30 min. at room temperature. The seeds were then slowly dried at 25 ºC for 2 days until they reached their original moisture content (∼7–9%) and stored until capsaicinoids content determinations and germination test were carried out [27,28]. Untreated seeds were used as control and subjected to the same disinfection, rinsing and drying conditions.

2.2.1. Digestion treatments.
To simulate the effect of the digestive tract of birds on breaking dormancy on Piquín chili seeds, a group of seeds were subjected to a chemical wet digestion process using HCl and H₂O₂. Seeds were dipped in 0.2 N HCl for 5 min., and rinsed with distilled deionized water (ddH₂O) for 2 min. Subsequently were oxidized with 0.5 N hydrogen peroxide for 5 min and newly rinsed with ddH₂O for 2 min. These redox sequence was repeated 3 times alternating both treatments.

2.2.2. Priming treatments.
Factorial halopriming was accomplished by imbibing 5 g of seed at 25 ºC in darkness for (24, 48 or 72 h) under an aerated solution of (KNO₃, K₂SO₄, NH₄NO₃, KCl, (NH₄)₂SO₄ or NH₄Cl) at -5, -10 or -15 atm (-0.5; -1.0 or -1.5 MPa respectively) of osmotic potential (Ψ) to prevent seeds from entering the phase III of hydration (growth) [27,29]. Solutions were prepared by dissolving different salts in 250 ml Erlenmeyer glasses containing 100 mL of distilled water [30]. Untreated seeds were used as control.

2.2.3. Priming integrated with gibberellic acid treatments.
Priming, integrated with GA₃ treatment [31], was performed using two of the priming treatments [KNO₃(-15 atm) and NH₄NO₃(-10 atm)], which further increased the germination parameters of the previous experiments. These priming treatments were supplemented with gibberellic acid (GA₃) at 100 or 200 ppm. Both controls (unprimed and without GA₃) were used as absolute and relative control respectively. Indices were calculated referring to absolute control (untreated seeds) and to their respective relative control (priming treatments) and these denoted with the subscript.
2.3. Capsaicinoids determination:

To test whether seeds capsaicinoids contents could be a contributor to seed germination, capsaicinoids content was determined on all seeds (primed and untreated) after treatments. Five-gram whole dry seeds were ground with a home blender for 3 minutes and then a fivefold volume of acetone was added, respectively, to the extract at 50 °C for 1 hour in triplicate. Centrifuged supernatant was taken for colorimetric analysis, following the methods proposed by Wang-Kyun et al [32].

2.4. Germination tests:

These were carried out in darkness in a temperature-controlled incubator held at 25 ± 0.5 °C and 100% relative humidity [33]. Seeds were placed on two layers of filter paper moistened with 3 mL of distilled water in covered 10 cm petri dishes. Germination values were recorded daily for 28 days to establish statistical data. From the total number of germinated seeds, final germination percentage (FGP) was calculated. For ungerminated seeds, tetrazolium chloride tests were conducted to differentiate between dormant and dead seeds [34]. Final latent percentage (FLP) and final mortality percentage (FMP) of seeds were calculated accordingly.

Primary root protrusion to 1 mm was scored as germinated seed. To evaluate root growth, a network of fiberglass of 1 mm² was placed under seeds. Primary root length (PRL) was measured in mm. Development germination index (DGI) allows to quantify effects (including FGP and PRL) of treatments (t) respect to control (o) on germination development. DGI was calculated by Zucconi tests [35] by following the formula: [DGI = 100·(FGP(t)/FGP(o)·(RL(t)/RL(o))] [36].

Days to 50% of FGP (T50) and days between 10% and 90% of FGP (G10–90) were also calculated. T50 is an inverse measure of mean germination rate, while G10–90 is an estimate of germination uniformity (GU) or germination synchrony [37]. To contrast the behavior of treatments (t) to control (o), these parameters were transformed in their respective indices, according to the following formulas: Rate germination index [RGI = 100·(T50(t)/T50(o))], synchrony germination index [SGI = 100·(G10–90(t)/G10–90(o))].

After germination testing, germinated seeds were transplanted to conventional seedling trays inside a greenhouse to evaluate the number of abnormal seedling generated by each treatment. Abnormal seedling percentage (ASP) and its corresponding abnormality seedling index [ASI= 100·(ASP(t)/ASP(o))], were calculated from abnormal plantlets.

2.5. Experimental design and statistical analysis:

Treatments were arranged in completely randomized design with four replications of 25 seeds. Data were subjected to multifactorial ANOVA test. Mean separation was performed by Fisher’s least significant difference (LSD0.05) test if F test was significant at p < 0.05 (*).

3. Results

Capsaicinoids contents, germination parameters, primary root growth and transplant abnormality for each seed treatment are shown in Tables 1, to 3 respectively. No differences were found between seeds lot or replications. The corresponding relative indexes, contrasting the behavior of each treatment with their control are also shown on Tables 1 to 3. The average daily percent germination values for treatments and control over a 28-day germination period are shown in Figure 1.
3.1. Digestion Treatments

Table 1 shows germination parameters of seeds digested with HCl and H$_2$O$_2$. Average values show no significant difference for CC, FLP, FMP, FGP, PRL, $T_{50}$, $G_{10-90}$, or ASP, while significant differences for DGI, RGI, SGI and ASI indices were found, indicating that these indices, are more sensitive to detect the treatment effects referred to control than the proper parameters. The chemical digestion of Piqúin pepper seeds does not affect capsaicinoids content (CC) on seeds. The lower FGP and PRL of digested seeds lead to a strong reduction on DGI (-33%) indicating a marked detrimental effect on germination development. Digestive treatments only increase mean germination rate (+11% RGI) and could contribute to break dormancy or latency reducing FLP (Table 1), but also reduces synchrony (-9% SGI), increases FMP, does not improve FGP, and strongly worsen early developmental stage of seedling and abnormality of transplants (+9% ASI).

3.2. Priming treatments

Average values of germination parameters and their indices are presented on Table 2. Significant differences were found in all factor of priming treatment (salt, time and $\Psi$) for all parameters and indices. As in previous analysis, indices are better to interpret and quantify the effect of treatments. Different behavior was observed for different salts, showing differences between K$^+$- and NH$_4^+$- salts on FGP (Fig. 1.) and between NO$_3^-$ and SO$_4^{2-}$ or Cl$^-$ on synchrony (Table 2). All treatment reduces capsaicinoids content on primed seeds. Highest CC reduction were obtained (Table 2) on seeds primed with NO$_3^-$ salts (more than SO$_4^{2-}$ or Cl$^-$) and at -10 or -15 atm (more than -5), for 48 or 72 h (more than 24).

FGP was increased 4-5 times and MGI reduced 44% for Cl$^-$ and SO$_4^{2-}$. NO$_3^-$ salts (of NH$_4^+$ or K$^+$) increased FGP (6 times) and reduced to ¼ seeds mortality (Table 2). A higher final percent of germinated seeds was also obtained for K$^+$ rather than NH$_4^+$ containing salts (Fig. 1). Highest FGP (together with low effect on PRL reduction) of NO$_3^-$ primed seeds lead to a strong increase on DGI, indicating a clear improvement on germinative process. DGI increases 3-4 times for Cl$^-$ and SO$_4^{2-}$ and by 5 times for NO$_3^-$. KNO$_3$ increased more than NH$_4$NO$_3$, not only DGI, but also RGI and SGI, whereas NH$_4$NO$_3$ reduced ASI more than KNO$_3$. An incremental effect was observed for priming time and $\Psi$ on FGP, DGI and RGI. Increments on germination rate were 6-12% higher using K$^+$ than NH$_4^+$ containing salts (Table 2). Latent seeds were only significantly reduced for K$_2$SO$_4$ or NH$_4$Cl salts at -10 or -15 atm for 48 or 72 h. Radicle length was only significantly reduced on KCl primed seeds under -5 atm of $\Psi$ for 24 or 48 h.

A differential effect was observed on germination synchrony for different factors. Germination synchrony increases on nitrate primed seeds, whereas was reduced on seeds primed with sulfate or chloride SGI. Priming times shorter than 72 h, or lower than -10 atm of $\Psi$ on priming solution, reduces synchrony (Table 2). Figure 1 shows the average percentage germination values over time for all priming and digestion treatments. A different behavior appears on the germination process for each treatment during 28 days of germination. Germination synchronies ($G_{10-90}$ and SGI on Table 2) were expanded by Cl$^-$ and SO$_4^{2-}$ whereas reduced by NO$_3^-$. Seeds primed with nitrate containing salts clearly increases germination synchrony and mean germination speed, but the effect is not indicted to be responsible for breaking of dormancy. Seeds latency (FLP) could probably be improved by including GA$_3$ in priming solutions (Fig. 1).
Abnormality of plantlets reduced as priming time increases and was lower for -10 atm of $\Psi$. ASI reduced 38% for Cl, 62% for $\text{SO}_2^-$ and 70% for $\text{NO}_3^-$. Graphic analysis of interactions (data not shown) indicated that 72 h priming treatments with $\text{NH}_4\text{NO}_3$ (-10 atm) and $\text{KNO}_3$ (-10 atm) are optimum regarding the improvement of PGI, MGI, DGI and ASI by adding AG3 to priming solutions.

### 3.3. Priming integrated with gibberellic acid treatments.

Average values of germination parameters and indices are presented on Table 3. All treatment significantly reduces capsaicinoids content on primed seeds. Highest CC reduction were obtained on seeds primed with $\text{NO}_3^-$ salts and at 200 ppm of AG3. Combined effects of nitrate priming and AG3 reduces initial capsaicinoids contents to 10%. An exponential correlation between CC and DGI were found (data not shown).

Pre-sowing with gibberellic acid treatments (Control +100 or +200ppm GA3) also shows (Fig. 1) a positive effect on germination respective to absolute control for all evaluated parameters (Table 3), except PRL (100 and 200ppm) and $G_{10-90}$ (100ppm).

GA3 significantly reduces latency (FLP) in Piquí n chili seeds (Table 3) referred to the absolute control and maintains this effect when it is added to priming solutions (Fig. 1). The addition of GA3 (at 100 or 200 ppm) activates dormant seeds to a rate between 73 and 84% respectively. This latency inhibition causes an increase in PGI of between 30 and 60%. However, GA3 additions to priming solutions increases FMP respect to their relative to controls.

GA3 significantly increases germination rate (RGI on Table 3) in respect of absolute or relative controls. At 200 ppm this RGI increase by 2.5 times. However, the effect of GA3 on synchrony is different. While 100ppm has no effect, additions of 200 ppm double the synchrony, reducing intense germination time from 12 to 8 days. These synergic effects of the addition of GA3 to priming solutions is clearly show for germination percentages on Figure 1. Conversely, 200 ppm GA3 has no effect on ASI, while 100 ppm GA3 significantly increases the presence of abnormal seedlings in primed seeds.

Gibberellic acid applied alone, significantly reduces the length of the primary root with respect to the absolute control. However, the integrated priming treatment with GA3, practically duplicate PRL for GA3 (200 ppm) and increases it by between 50 and 70% for GA3 (100 ppm). These increases in PRL together with the originated in FGP lead to double or triple values of DGI (associated with GA3) compared to their respective relative controls. On the other hand, the reduction in PRL (associated with the application of GA3) regarding the absolute control, neutralizes the positive impact generated on FGP and originates DGI increases, on relative control, like those produced by the halopriming without GA3.

### 3.4. Figures, Tables and Schemes

#### Table 1. Average values, ANOVA significance and LSD0.05 values of *Capsicum annuum* var. *glabriusculum* seeds and seedling, germinated in darkness at 25 °C following digestion treatments.

<table>
<thead>
<tr>
<th></th>
<th>CC (µg·g⁻¹)</th>
<th>FLP (%)</th>
<th>FMP (%)</th>
<th>FGP (%)</th>
<th>PRL (mm)</th>
<th>DGI</th>
<th>T50 (d)</th>
<th>RGI</th>
<th>$G_{10-90}$ (d)</th>
<th>SGI (%)</th>
<th>ASP (%)</th>
<th>ASI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Significance</strong></td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td>*</td>
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<tr>
<td>Control seeds</td>
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<td>43.9</td>
<td>8.1</td>
<td>25.4</td>
<td>100b</td>
<td>25.2</td>
<td>100a</td>
<td>14.1</td>
<td>100b</td>
<td>15.1</td>
<td>100a</td>
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<td>Digested seeds</td>
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<td>45.0</td>
<td>46.4</td>
<td>7.7</td>
<td>17.5</td>
<td>66a</td>
<td>23.5</td>
<td>111b</td>
<td>14.3</td>
<td>91a</td>
<td>15.9</td>
<td>114b</td>
</tr>
<tr>
<td><strong>LSD0.05</strong></td>
<td>260</td>
<td>3.63</td>
<td>4.01</td>
<td>0.66</td>
<td>10.98</td>
<td>13.9</td>
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<td>5.91</td>
<td>1.18</td>
<td>4.71</td>
<td>1.20</td>
<td>9.84</td>
</tr>
</tbody>
</table>

Capsaicinoids Content (CC); Final Latent Percentage (FLP); Final Mortality Percentage (FMP); Final Germination Percentage (FGP); Primary Root Length (PRL); Development Germination Index (DGI); Days to 50% of FGP
(T50); Rate Germination Index (RGI); Days between 10% and 90% of FGP (G_{10-90}); Synchrony Germination Index (SGI); Abnormal Seedling Percentage (ASP); Abnormality Seedling Index (ASI).

Means within the same column followed by the same letter are not different at p ≤ 0.05 per Fisher’s least significant difference test.

NS, * Nonsignificant or significant differences at p ≤ 0.05.

### Table 2. Average values, ANOVA significance and LSD_{0.05} values of *Capsicum annuum* var. *glabriusculum* seeds

and seedling, germinated in darkness at 25°C following priming (Pr) treatments.

<table>
<thead>
<tr>
<th>Pr salt</th>
<th>CC (µg·g⁻¹)</th>
<th>FLP (%)</th>
<th>FMP (%)</th>
<th>FGP (%)</th>
<th>PRL (mm)</th>
<th>DGI</th>
<th>T₅₀ (d)</th>
<th>RGI</th>
<th>G_{10-90} (d)</th>
<th>SGI</th>
<th>ASP (%)</th>
<th>ASI</th>
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<tbody>
<tr>
<td>Control</td>
<td>957c</td>
<td>44.7c</td>
<td>48.8c</td>
<td>7.9a</td>
<td>18.3c</td>
<td>100a</td>
<td>25.5e</td>
<td>100a</td>
<td>14.6c</td>
<td>100a</td>
<td>14.8c</td>
<td>100c</td>
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<tr>
<td>NH₄Cl</td>
<td>638c</td>
<td>39.2ab</td>
<td>26.2b</td>
<td>36.5c</td>
<td>17.1abc</td>
<td>421bc</td>
<td>22.4d</td>
<td>113b</td>
<td>21.1f</td>
<td>67a</td>
<td>12.9bc</td>
<td>87bc</td>
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<td>(NH₄)₂SO₄</td>
<td>469b</td>
<td>39.9abc</td>
<td>28.0b</td>
<td>32.4b</td>
<td>16.8abc</td>
<td>366bc</td>
<td>22.1cd</td>
<td>115bc</td>
<td>22.1g</td>
<td>64a</td>
<td>11.4b</td>
<td>77b</td>
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<td>45.0d</td>
<td>16.8abc</td>
<td>501de</td>
<td>22.1d</td>
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<td>360b</td>
<td>21.0b</td>
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<td>100a</td>
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<td>100a</td>
<td>14.8c</td>
<td>100c</td>
</tr>
<tr>
<td>-5</td>
<td>555b</td>
<td>46.2c</td>
<td>20.9a</td>
<td>34.0b</td>
<td>15.5a</td>
<td>368b</td>
<td>22.2c</td>
<td>117b</td>
<td>18.2c</td>
<td>83a</td>
<td>14.3c</td>
<td>97c</td>
</tr>
<tr>
<td>-10</td>
<td>395a</td>
<td>38.7b</td>
<td>21.8ab</td>
<td>39.7c</td>
<td>17.3b</td>
<td>462c</td>
<td>21.2b</td>
<td>118b</td>
<td>17.0bc</td>
<td>91ab</td>
<td>8.8a</td>
<td>55a</td>
</tr>
<tr>
<td>-15</td>
<td>428a</td>
<td>34.5a</td>
<td>24.8b</td>
<td>40.3c</td>
<td>18.1b</td>
<td>496c</td>
<td>19.8a</td>
<td>126c</td>
<td>16.0ab</td>
<td>96b</td>
<td>10.8b</td>
<td>77b</td>
</tr>
</tbody>
</table>

Capsaicinoids Content (CC); Final Latent Percentage (FLP); Final Mortality Percentage (FMP); Final Germination Percentage (FGP); Primary Root Length (PRL); Development Germination Index (DGI); Days to 50% of FGP (T₅₀); Rate Germination Index (RGI); Days between 10% and 90% of FGP (G_{10-90}); Synchrony Germination Index (SGI); Abnormal Seedling Percentage (ASP); Abnormality Seedling Index (ASI).

Means within the same column followed by the same letter are not different at p ≤ 0.05 per Fisher’s least significant difference test.

* Significant differences at p ≤ 0.05.
Table 3. Average values, ANOVA significance and LSD values of *Capsicum annum* var. *glabriusculum* seeds and seedling, germinated in darkness at 25 °C following presowing with gibberellic acid treatments and priming integrated with gibberellic acid treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CC (µg·g⁻¹)</th>
<th>FLP (%)</th>
<th>FMP (%)</th>
<th>FGP (%)</th>
<th>PRL (mm)</th>
<th>DGI</th>
<th>T₅₀ (d)</th>
<th>RGI</th>
<th>G₁₀–₉₀ (d)</th>
<th>SGI</th>
<th>ASP (%)</th>
<th>ASI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>824e</td>
<td>47.0d</td>
<td>45.4f</td>
<td>7.6a</td>
<td>21.4c</td>
<td>100a</td>
<td>26.7f</td>
<td>100a</td>
<td>14.9de</td>
<td>100a</td>
<td>32.0f</td>
<td>100f</td>
</tr>
<tr>
<td>+100 ppm GA₁³</td>
<td>491d</td>
<td>11.9b</td>
<td>27.1e</td>
<td>60.1d</td>
<td>13.4a</td>
<td>545b</td>
<td>10.8bc</td>
<td>214b</td>
<td>15.6e</td>
<td>168a</td>
<td>21.4e</td>
<td>71e</td>
</tr>
<tr>
<td>+200 ppm GA₁³</td>
<td>384c</td>
<td>7.1a</td>
<td>16.9c</td>
<td>76.0f</td>
<td>13.7a</td>
<td>674c</td>
<td>8.2a</td>
<td>245c</td>
<td>8.8a</td>
<td>297c</td>
<td>15.7d</td>
<td>52d</td>
</tr>
<tr>
<td>NH₄NO₃ (100atm)</td>
<td>461d</td>
<td>43.4c</td>
<td>8.2ab</td>
<td>48.4b</td>
<td>18.0b</td>
<td>579b</td>
<td>22.8e</td>
<td>94a</td>
<td>12.9c</td>
<td>202b</td>
<td>6.9a</td>
<td>23a</td>
</tr>
<tr>
<td>+100 ppm GA₁³</td>
<td>201b</td>
<td>12.0b</td>
<td>23.4d</td>
<td>64.6e</td>
<td>30.9d</td>
<td>1331d</td>
<td>11.3e</td>
<td>192b</td>
<td>15.5e</td>
<td>168a</td>
<td>20.6e</td>
<td>69ec</td>
</tr>
<tr>
<td>+200 ppm GA₁³</td>
<td>74a</td>
<td>12.2b</td>
<td>16.4c</td>
<td>76.9f</td>
<td>39.8e</td>
<td>2036e</td>
<td>8.0a</td>
<td>262c</td>
<td>8.0a</td>
<td>328d</td>
<td>7.0a</td>
<td>23a</td>
</tr>
<tr>
<td>KNO₃ (150atm)</td>
<td>402c</td>
<td>42.4c</td>
<td>5.4a</td>
<td>52.3c</td>
<td>19.1b</td>
<td>665c</td>
<td>18.4d</td>
<td>119a</td>
<td>10.2b</td>
<td>254b</td>
<td>11.2b</td>
<td>37b</td>
</tr>
<tr>
<td>+100 ppm GA₁³</td>
<td>209b</td>
<td>6.7a</td>
<td>21.6d</td>
<td>66.3e</td>
<td>28.9d</td>
<td>1276d</td>
<td>10.1b</td>
<td>210b</td>
<td>14.2d</td>
<td>183a</td>
<td>13.2c</td>
<td>44c</td>
</tr>
<tr>
<td>+200 ppm GA₁³</td>
<td>72a</td>
<td>6.8a</td>
<td>11.5b</td>
<td>81.8g</td>
<td>38.4e</td>
<td>2096e</td>
<td>8.1a</td>
<td>269c</td>
<td>8.1a</td>
<td>323d</td>
<td>11.1b</td>
<td>37b</td>
</tr>
</tbody>
</table>

Capsaicinoids Content (CC); Final Latent Percentage (FLP); Final Mortality Percentage (FMP); Final Germination Percentage (FGP); Primary Root Length (PRL); Development Germination Index (DGI); Days to 50% of FGP (T₅₀); Rate Germination Index (RGI); Days between 10% and 90% of FGP (G₁₀–₉₀); Synchrony Germination Index (SGI); Abnormal Seedling Percentage (ASP); Abnormality Seedling Index (ASI).

Means within the same column followed by the same letter are not different at p ≤ 0.05 per Fisher’s least significant difference (LSD) test.

* Significant differences at p ≤ 0.05.
Figure 1. Cumulative germination percentage of Piquín pepper after seeds treatments (digested or primed) monitored for 28 days. Error bars are presented only for control, digested and KNO₃ treatments.
4. Discussion

4.1. Digestion Treatment.
While some authors argue that Piquín chili seed germination increases after passage through the digestive tract of birds, evidence of this fact has not been provided [2,10]. Digestive treatments could contribute to breaking dormancy, increasing mean germination speed, but do not improve germination percentage or synchrony and strongly worsen early developmental stage of seedlings. The positive effects seen on germination related to birds appear to be more associated to the dispersal and deposition of seeds in favorable environments that stimulate further germination [38]. Digestion treatments have not shown any positive effect on the germination of Piquín chili seeds of. Authors have presented both similar results [39], and have also found large differences [11,33,40] in the behavior of different accessions of plants.

4.2. Priming treatments.
Priming has been proposed as a mechanism of invocation of different stress tolerance of germinating seeds [18]. Seed priming treatments have been applied to various crops under saline conditions [41,42]. Some authors find that a specific ion or salt is not essential in priming pepper seed [43], and other horticultural crop species [15]. Nitrate enhanced germination and seedling establishment rates, under adverse conditions, of onion [44] tomato and asparagus [45], melon [46], watermelon [47], husk tomato [30] and pepper [48,49]. Our results also indicate that nitrate-containing salts are more efficient than nitrate-free salts at promoting germination (except breaking dormancy) of primed seeds. In addition, the effects of priming with KNO$_3$ seem to be more positive than NO$_3$NO$_3$ on main germination and establishment of seedling parameters (except for seed mortality and seedling abnormality). Seed priming stimulates the pre-germination metabolic processes and prepares the seed for primary root protrusion. It increases the antioxidant system activity and the repair of membranes, moreover, the reduction of capsaicinoids on seeds during priming, could contribute to break dormancy and stimulate germinative process on primed seeds. These changes promote seed vigour during germination and emergence [17]. Time-course experiments show that effective priming is strongly dependent on both the osmotic potential of the priming solution and the duration of the treatment to avoid “overpriming” [43]. Accordingly, the recommended values of osmotic potential to improve the Piquín chili seed germination must be between -10 and -15 atm (-1.0 and -1.5 MPa), the treatment time must be between 48 and 72 h.

A small number of Piquín pepper studies, very heavily dependent on the origin of seeds accessions and genetic diversity, presented conflicting results [8,26,31,33,50]. Authors do not find positive effects of KNO$_3$ priming, whereas only see positive effects with GA$_3$ at extremely high doses (5000 ppm). However, none of these studies combine priming with GA$_3$ at low doses. The undesirable observed effects of seed latency (LGI), mean germination rate (RGI) and synchrony (SGI), could be improved by including gibberellic acid (GA$_3$) in priming solutions (as shown in Figure 1).

4.3. Priming integrated with gibberellic acid treatments.
Halopriming with the addition of plant growth regulators may be an effective way to shorten emergence time and increase stand establishment in watermelon [25] and pepper at low temperatures
Halo-priming using KNO₃ or a growth regulator like GA₃ improves the rate of germination and reduces the mean germination time in endive and chicory [24]. The integration of priming with GA₃ was effective in improving germination and establishment of pepper and tomato seeds. Priming, during which germination is suspended, provides an unique way to rapidly and efficiently digest the endosperm by GA-induced enzymes and reduce the mechanical restraints of endosperm thus providing energy to start and sustain embryo growth [51]. Studies of genetics and physiology have shown the important roles of the plant hormones such as abscisic acid and gibberellin in the regulation of seed dormancy and germination [52]. Considerable improvements in seed germination performance, an increase in rate and uniformity, and emergence and establishment of seedlings are shown for KNO₃ and GA₃ treatments, in agreement with Tzortzakis [24]. The lowest values of capsaicinoids found on KNO₃ primed seeds together with AG₃ could reduce the allelopathic effect on pepper seed germination. Since high concentrations of capsaicin inhibit the germination of chili seeds [3], the positive effects on germination may be due to the elimination of these as germination inhibitors [9,26]. Finally, our results provide essential information needed for the development of guidelines for the domestication and cultivation of Piquín chili plants.

5. Conclusions

This study showed that it is possible to improve dormancy and germination processes on Piquín chili seeds by priming techniques. Wild Piquín chili seed primed with KNO₃ (10 atm; 72h) integrated with GA₃ (200ppm) reduced time to germination start (dormancy) and improved germination parameters. Moreover, the results of current study provide essential information needed for the development of guidelines for the domestication and cultivation of Piquín chili plants.

Author Contributions:

MFQ and MG. conceived and designed the experiments; OG and AGC performed the experiments; MFQ, PD, JM and MG. analyzed the data; MFQ, PD, JM AGC and MG. contributed reagents/materials/analysis tools; MFQ and MG wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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