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Article

Improving dormancy and 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 pre-sowing treatment of seeds: a) chemical seeds digestion; b) halopriming (with K^+ or NH_4^+ of NO_3^-, SO_4^{2-} or Cl^-) at different priming times (24, 48 or 72 h) and osmotic potential (-5, -10 or -15 atm) and c) previously selected halopriming (KNO_3 and NH_4NO_3) + Gibberellic acid (GA_3, at 100 or 200 ppm) were tested. Digestion treatments did show a negative effect on seed germination. Recommended values of osmotic potential (Ψ_s), 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. KNO_3 and secondly GA_3 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

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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 [5,6]. While there is basic information that allows for developing guidelines for its cultivation [7], 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 [7–9]. Some authors suggest that germination of its seeds is restricted by physiological dormancy [10] and is achieved after passing through the digestive tract of certain birds [11]. Seeds of many species remain viable after passing through the digestive tracts of animals, with varying effects on germination [12]. 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 [13,14].

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) [15]. 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 [14,16]. It also involves complex physiological and biochemical process which offers an effective means to improve seed quality [17], seed germination and vigor [18]. Priming treatments are widely applied by seed companies to increase the germination rate and uniformity of seedling establishment of commercial vegetable and flower seeds [19,20]. Primed seeds are equipped with advanced germination and exhibit improved germination rate and uniformity [21]. The benefits, associated with certain physiological, biochemical, cellular and molecular changes [19], include rapid, uniform and increased germination, improved seedling vigor and growth under a broad range of environments resulting in better stand establishment [22–25]. Different priming treatments such as hydropriming (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 [14,26,27]. Halopriming can affect osmoregulation in seeds by the active uptake of inorganic ions, promoting K^+ and Ca^{2+} 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 [19]. 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 [28]. 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 [29–32], and other vegetables [33,34].

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 (GA_3) treatments. This information is

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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 [35,36].

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 (ddH_2O) 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. [36,37]. 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 digestion process using HCl and H_2O_2. Seeds were dipped in 0.2 N HCl for 5 min., and rinsed with distilled deionized water (ddH_2O) for 2 min. Subsequently were oxidized with 0.5 N hydrogen peroxide for 5 min and newly rinsed with ddH_2O for 2 min.

2.2.3. Priming treatments.

Factorial halopriming was accomplished by imbibing 5 g of seed at 25 °C in darkness for (24, 48 or 72 h) under an aerated solution of (KNO_3, K_2SO_4, NH_4NO_3, KCl, $(NH_4)_2SO_4$ or NH_4Cl) at -5, -10 or -15 atm (-0.5; -1.0 or -1.5 MPa respectively) of osmotic potential (Ψ_s) to prevent seeds from entering the phase III of hydration (growth) [19,36,38]. Solutions were prepared by dissolving different salts in 250 ml Erlenmeyer glasses containing 100 mL of distilled water [39]. Untreated seeds were used as control.

2.2.3. Priming integrated with gibberellic acid treatments.

Priming, integrated with GA_3 treatment [40], was performed using two of the priming treatments [$KNO_{3\,(-15\,atm)}$ and $NH_4NO_{3\,(-10\,atm)}$], which further increased the germination parameters of the previous experiments. These priming treatments were supplemented with gibberellic acid (GA_3) at 100 or 200 ppm. Both controls (unprimed and without GA_3) 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.

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23. 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 ºC for 1 hour in triplicate. Centrifuged supernatant was taken for colorimetric analysis, following the methods proposed by Wang-Kyun *et al* [41].

2.4. Germination tests:

These were carried out in darkness in a temperature-controlled incubator held at 25 ± 0.5 °C and 100% RH [42]. 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 [43]. Final latent percentage (FLP) and final mortality percentage (FMP) of seeds were calculated accordingly.

Primary root protrusion to 1 mm was scored as germination. To evaluate root growth, a network of fiberglass of 1 mm^2 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 [44] by following the formula: [$DGI = 100 \cdot (FGP_{(t)}/FGP_{(o)} \cdot (RL_{(t)}/RL_{(o)})$] [45,46].

Days to 50% of FGP (G_{50}) and days between 10% and 90% of FGP ($G_{10\text{–}90}$) were also calculated. G_{50} is an inverse measure of mean germination rate, while $G_{10\text{–}90}$ is an estimate of the spread of germination, the inverse of germination synchrony [47]. 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 [$RGI = 100 \cdot (G_{50(o)}/G_{50(t)})$]; synchrony germination index [$SGI = 100 \cdot (G_{10\text{–}90(o)}/G_{10\text{–}90(t)})$].

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 [$ASI = 100 \cdot (ASP_{(t)}/ASP_{(o)})$], 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 ($LSD_{0.05}$) 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

 doi:10.20944/preprints201802.0160.v1

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_2O_2. Average values show no significant difference for CC, FLP, FMP, FGP, PRL, G_{50}, $G_{10\text{-}90,}$ 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 Piquín 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 Ψ_s) 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_4NO_3, not only DGI, but also RGI and SGI, whereas NH_4NO_3 reduced ASI more than KNO_3. An incremental effect was observed for priming time and Ψ_s 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_2SO_4 or NH_4Cl 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 Ψ_s 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 Ψ_s 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 ($G_{10\text{-}90}$ 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

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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 Ψ_s. ASI reduced 38% for Cl^-, 62% for SO_4^{2-} and 70% for NO_3^-. Graphic analysis of interactions (data not shown) indicated that 72 h priming treatments with $NH_4NO_{3\,(-15atm)}$ and $KNO_{3\,(-10atm)}$ are optimum regarding the improvement of PGI, MGI, DGI and ASI by adding AG_3 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 NO_3^- salts and at 200 ppm of AG_3. 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 GA_3) 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 $G_{10\text{-}90}$ (100ppm).
GA_3 significantly reduces latency (FLP) in Piquín 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 GA_3 (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, GA_3 additions to priming solutions increases FMP respect to their relative to controls.
GA_3 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 GA_3 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 GA_3 to priming solutions is clearly show for germination percentages on Figure 1. Conversely, 200 ppm GA_3 has no effect on ASI, while 100 ppm GA_3 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 GA_3, practically duplicate PRL for $GA_{3\,(200\ ppm)}$ and increases it by between 50 and 70% for $GA_{3\,(100\ ppm)}$. These increases in PRL together with the originated in FGP lead to double or triple values of DGI (associated with GA_3) compared to their respective relative controls. On the other hand, the reduction in PRL (associated with the application of GA_3) 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 GA_3.

3.4. Figures, Tables and Schemes

Table 1. Average values, ANOVA significance and $LSD_{0.05}$ values of *Capsicum annuum* var. *glabriusculum* seeds and seedless, germinated in darkness at 25 °C following digestion treatments.

	CC ($\mu g{\cdot}g^{-1}$)	FLP (%)	FMP (%)	FGP (%)	PRL (mm)	DGI	G_{50} (d)	RGI	$G_{10\text{-}90}$ (d)	SGI	ASP (%)	ASI
Significance	NS	NS	NS	NS	NS	*	NS	*	NS	*	NS	*
Control seeds	973	46.3	43.9	8.1	25.4	100b	25.2	100a	14.1	100b	15.1	100a
Digested seeds	1007	45.0	46.4	7.7	17.5	66a	23.5	111b	14.3	91a	15.9	114b
$LSD_{0.05}$	260	3.63	4.01	0.66	10.98	13.9	2.04	5.91	1.18	4.71	1.20	9.84

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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 (G_{50}); Rate Germination Index (RGI); Days between 10% and 90% of FGP (G_{10-90}); Synchrony Germination Index (SGI); Abnormal Seedless Percentage (ASP); Abnormality Seedless Index (ASI).

Means within the same column followed by the same letter are not different at $p \leq 0.05$ per Fisher's least significant difference test.

NS, * Nonsignificant or significant differences at $p \leq 0.05$.

Table 2. Average values, ANOVA significance and $LSD_{0.05}$ values of *Capsicum annuum* var. *glabriusculum* seeds and seedless, germinated in darkness at 25°C following priming (Pr) treatments.

	CC (μg·g-1)	FLP (%)	FMP (%)	FGP (%)	PRL (mm)	DGI	G_{50} (d)	RGI	G_{10-90} (d)	SGI	ASP (%)	ASI
Pr salt	*	*	*	*	*	*	*	*	*	*	*	*
Control	957d	44.7c	48.8c	7.9a	18.3c	100a	25.5e	100a	14.6c	100d	14.8c	100c
NH_4Cl	638c	39.2ab	26.2b	36.5c	17.1abc	421bc	22.4d	113b	21.1f	67a	12.9bc	87bc
$(NH_4)_2SO_4$	469b	39.9abc	28.0b	32.4b	16.8abc	366bc	22.1cd	115bc	22.1g	64a	11.4b	77b
NH_4NO_3	319a	43.2bc	11.9a	45.0d	16.8abc	501de	21.0b	120cd	12.0b	120e	8.6a	58a
KCl	579c	39.7ab	28.2b	32.7bc	16.0a	360b	21.1bc	120cd	17.1d	83c	12.8bc	86bc
K_2SO_4	441b	36.8a	27.2b	35.3bc	17.6abc	437cd	20.8b	121d	18.9e	75b	11.7b	79b
KNO_3	309a	40.2abc	13.5a	46.1d	17.8bc	565e	19.0a	132e	11.0a	132f	10.1ab	72ab
Pr time (h)	*	*	*	*	*	*	*	*	*	*	*	*
0	957c	44.7c	48.8d	7.9a	18.3c	100a	25.5d	100a	14.6a	100c	14.8c	100c
24	607b	49.7d	16.9a	33.7b	15.2a	353b	22.7c	112b	18.9c	78a	13.8c	93c
48	420a	38.3b	23.7b	38.5c	16.6b	433c	21.3b	118c	17.2b	89b	11.6b	80b
72	350a	31.5a	26.8c	41.8d	19.2c	538d	19.2a	131d	15.0a	103c	8.4a	57a
Pr Ψ_o (atm)	*	*	*	*	*	*	*	*	*	*	*	*
0	957c	44.7c	48.8c	7.9a	18.3b	100a	25.5d	100a	14.6a	100b	14.8c	100c
-5	555b	46.2c	20.9a	34.0b	15.5a	368b	22.2c	117b	18.2c	83a	14.3c	97c
-10	395a	38.7b	21.8ab	39.7c	17.3b	462c	21.2b	118b	17.0bc	91ab	8.8a	55a
-15	428a	34.5a	24.8b	40.3c	18.1b	496c	19.8a	126c	16.0ab	96b	10.8b	77b

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 (G_{50}); Rate Germination Index (RGI); Days between 10% and 90% of FGP (G_{10-90}); Synchrony Germination Index (SGI); Abnormal Seedless Percentage (ASP); Abnormality Seedless Index (ASI).

Means within the same column followed by the same letter are not different at $p \leq 0.05$ per Fisher's least significant difference test.

* Significant differences at $p \leq 0.05$.c

Table 3. Average values, ANOVA significance and $LSD_{0.05}$ values of *Capsicum annuum* var. *glabriusculum* seeds and seedless, germinated in darkness at 25 °C following presowing with gibberellic acid treatments and priming integrated with gibberellic acid treatments.

	CC (μg·g-1)	FLP (%)	FMP (%)	FGP (%)	PRL (mm)	DGI	G_{50} (d)	RGI	G_{10-90} (d)	SGI	ASP (%)	ASI
Treatment	*	*	*	*	*	*	*	*	*	*	*	*
Control	824e	47.0d	45.4f	7.6a	21.4c	100a	26.7f	100a	14.9de	100a	32.0f	100f
+100ppm GA_3	491d	11,9b	27.1e	60.1d	13.4a	545b	10.8bc	214b	15.6e	168a	21.4e	71e
+200ppm GA_3	384c	7.1a	16.9c	76.0f	13.7a	674c	8.2a	245c	8.8a	297c	15.7d	52d
NH_4NO_3 (-10atm)	461d	43.4c	8.2ab	48.4b	18.0b	579b	22.8e	94a	12.9c	202b	6.9a	23a
+100 ppm GA_3	201b	12,0b	23.4d	64.6e	30.9d	1331d	11.3c	192b	15.5e	168a	20.6e	69ec

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+200 ppm GA_3	74a	12.2b	16.4c	76.9f	39.8e	2036e	8.0a	262c	8.0a	328d	7.0a	23a
KNO_3 (-15atm)	402c	42.4c	5.4a	52.3c	19.1b	665c	18.4d	119a	10.2b	254b	11.2b	37b
+100 ppm GA_3	209b	6.7a	21.6d	66.3e	28.9d	1276d	10.1b	210b	14.2d	183a	13.2c	44c
+200 ppm GA_3	72a	6.8a	11.5b	81.8g	38.4e	2096e	8.1a	269c	8.1a	323d	11.1b	37b

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 (G_{50}); Rate Germination Index (RGI); Days between 10% and 90% of FGP (G_{10-90}); Synchrony Germination Index (SGI); Abnormal Seedless Percentage (ASP); Abnormality Seedless Index (ASI).

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

* Significant differences at $p \leq 0.05$.

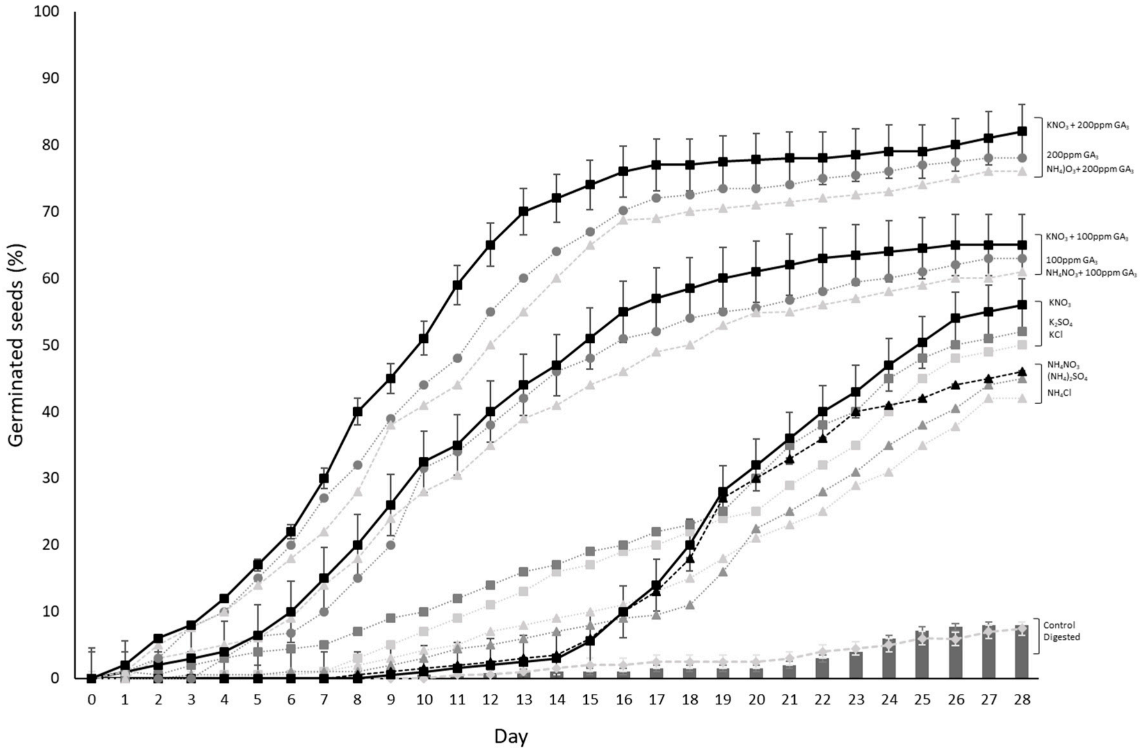

Figure 1. Germination percentage of Piquin pepper after seeds treatments (digested or primed) monitored for 28 days. Error bars are presented only for control, digested and KNO_3 treatments.

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 [2,11]. 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 [7,48,49]. Digestion treatments have not shown any positive effect on the germination of Piquín chili seeds of.

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Authors have presented both similar results [50], and have also found large differences [12,42,51–53] 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 [21,54]. Seed priming treatments have been applied to various crops under saline conditions [19,55–57]. Some authors find that a specific ion or salt is not essential in priming pepper seed [58], and other horticultural crop species [17]. Nitrate enhanced germination and seedling establishment rates, under adverse conditions, of onion [59] tomato and asparagus [16], melon [60], watermelon [61,62], husk tomato [39] and pepper [63–66]. 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_4NO_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 [19].

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" [58,67,68]. 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 [9,35,40,42,69,70]. 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 [34] and pepper at low temperatures [47]. Halo-priming using KNO_3 or a growth regulator like GA_3 improves the rate of germination and reduces the mean germination time in endive and chicory [33].

The integration of priming with GA_3 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 [30]. 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 [71].

Considerable improvements in seed germination performance, an increase in rate and uniformity, and emergence and establishment of seedlings are shown for KNO_3 and GA_3 treatments, in

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agreement with Tzortzakis [33]. The lowest values of capsaicinoids found on KNO_3 primed seeds together with AG_3 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 [10,35]. 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 Piquin chili seeds by priming techniques. Wild Piquin chili seed primed with $KNO_{3\,(-10atm;\,72h)}$ integrated with $GA_{3\,(200ppm)}$ reduced time to germination start (dormancy) and improved germination parameters. Moreover, the study results provide essential information needed for the development of guidelines for the domestication and cultivation of Piquín chili plants.

Author Contributions:

"X.X. and Y.Y. conceived and designed the experiments; X.X. performed the experiments; X.X. and Y.Y. analyzed the data; W.W. contributed reagents/materials/analysis tools; Y.Y. wrote the paper.

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