1   *Article*

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

5   **Maria Fernanda Quintero C [1],*, Oscar Guillen Castillo [1], Pablo Delgado Sánchez [1], José Marín**
6   **Sánchez [1], Ana Isabel Guzmán [2] and José Miguel Guzmán [2]***

7   [1]   Faculty of Agronomy and Veterinary, University of San Luis Potosi. Km. 14.5 Carretera San Luis Potosí,
8   Matehuala, Ejido Palma de la Cruz, Soledad de Graciano Sánchez, San Luis Potosí, S.L.P. CP. 78321.
9   maria.quintero@uaslp.mx (MFQ); oscarguillen_castillo@hotmail.com (OG); pablo.delgado@uaslp.mx (PD);
10   jose.marin@uaslp.mx (JM)
11   [2]   Departamento de Agronomía, Universidad de Almería, Campus Excelencia Internacional Agroalimentaria
12   (cei A3). España. anais_9333@hotmail.com (AGC);   mguzman@ual.es; mguzman@ual.es (MG)
13   *   Correspondence: maria.quintero@uaslp.mx

14   Received: date; Accepted: date; Published: date

15   **Abstract:** The effects of different priming techniques were evaluated to improve the dormancy and
16   germination of wild seeds of "Piquín" chili pepper. Three experiments were designed for pre-
17   sowing treatment of seeds: a) chemical seeds digestion; b) halopriming (with K^+ or NH_4^+ of NO_3^-,
18   SO_4^{2-} or Cl^-) at different priming times (24, 48 or 72 h) and osmotic potential (-5, -10 or -15 atm) and
19   c) previously selected halopriming (KNO_3 and NH_4NO_3) + Gibberellic acid (GA_3, at 100 or 200 ppm)
20   were tested. Digestion treatments did show a negative effect on seed germination. Recommended
21   values of osmotic potential (Ψ_s), to improve Piquín chili seed germination, must be between -10 and
22   -15 atm (-1.0 and -1.5 MPa) and the priming time must be between 48 and 72 hours. Priming
23   techniques can considerably reduce Capsaicinoids content on seeds, improve dormancy, seed
24   germination performance, and increase the rate and uniformity of seedling establishment. KNO_3
25   and secondly GA_3 treatments may improve rapid and uniform germination and seedling
26   emergence. The results provide basic information to develop guidelines for commercial
27   establishment of Piquín pepper crops.

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

30

31

32 1. Introduction

33   Chili "Piquín" or "chiltepín", [*Capsicum annuum* var. *glabriusculum* (Dunal) Heiser & Pickersgill); syn.
34   *C. annuum* var. *aviculare (Dierb.) D'Arcy & Eshbaugh*)], is distributed from Colombia, Central America,
35   and Mexico to the southwestern United States. The natural populations of "chiltepín" are considered
36   an important genetic resource for pepper crop improvement [1]. This species is of great significance
37   in the culture and identity of indigenous peoples of Mexico who usually harvested its fruits of wild
38   plants [2]. The heat of chili pepper is due to the accumulation of capsaicinoids, a group of related
39   alkaloids unique to *Capsicum*. Capsaicinoids are produced in the fruit placenta and transferred to the
40   seeds during fruit maturation [3]. In highland regions where it occurs, is an important part of the

41   local economy, especially in the time of harvest, generating employment and income for rural
42   communities. This activity might threaten the genetic diversity in this species, affecting habitat
43   degradation of natural populations of wild pepper [4]. This problem could be solved by limiting the
44   collection of wild populations and increasing their cultivation as a crop, in turn generating economic
45   resources derived from this activity [5]. While there is basic information that allows for developing
46   guidelines for its cultivation [6], more research, related to germination, stand establishment and crop
47   development and productivity, is necessary to develop commercial Piquín pepper crops.
48   Domestication of Piquín pepper plants have not been fully developed because problems are
49   encountered related to low and erratic seed germination, morphologic and genetic variability, and
50   limited environmental physiology information [6–8]. Some authors suggest that germination of its
51   seeds is restricted by physiological dormancy [9] and is achieved after passing through the digestive
52   tract of certain birds [10]. Seeds of many species remain viable after passing through the digestive
53   tracts of animals, with varying effects on germination [11]. Seed dormancy is generally an undesirable
54   characteristic in agricultural crops, where rapid germination and growth are required. Extensive
55   domestication and breeding of crop species have ostensibly removed most dormancy mechanisms
56   present in the seeds of their wild ancestors. Studies have reported a myriad of methods to break seed
57   dormancy, including chemical, mechanical, thermal, and hormonal seed treatments [12,13].
58   The beneficial effects of priming on the vigor, germination of seeds and establishment of the seedlings
59   is known since the times of Pliny the elder (A.D. 23-79) [14]. Seed priming is a presowing treatment
60   involves the controlled hydration of seeds, sufficient to allow pregerminative metabolic events to
61   take place but insufficient to allow primary root protrusion through the seed coat [13]. It also involves
62   complex physiological and biochemical process which offers an effective means to improve seed
63   quality [15], seed germination and vigor [16]. Priming treatments are widely applied by seed
64   companies to increase the germination rate and uniformity of seedling establishment of commercial
65   vegetable and flower seeds [17]. Primed seeds are equipped with advanced germination and exhibit
66   improved germination rate and uniformity [18]. The benefits, associated with certain physiological,
67   biochemical, cellular and molecular changes [17], include rapid, uniform and increased germination,
68   improved seedling vigor and growth under a broad range of environments resulting in better stand
69   establishment [19]. Different priming treatments such as hydropriming (soaking in water),
70   halopriming (soaking in inorganic salt solutions), osmopriming (soaking in solutions of different
71   organic osmotic molecules), thermopriming (treatment of seed with low or high temperatures) or
72   solid matrix priming (treatment of seed with solid matrices) can be effectively employed to prime a
73   large number of hot pepper seeds at one time [13,20]. Halopriming can affect osmoregulation in seeds
74   by the active uptake of inorganic ions, promoting K^+ and Ca^{2+} absorption and decreasing Na^+ and Cl^-
75   accumulation. Potassium plays an important role in balancing membrane potential and turgor,
76   activating enzymes, and regulating osmotic pressure in cells [17]. Some authors hypothesized that
77   capsaicinoids could have some allelopathic effect on pepper seed germination [3]. Capsaicinoids are
78   a well-established allelochemical and has been shown to reduce root and shoot growth or suppress
79   germination in several plant species [21]. The effects of incorporating plant growth regulators into
80   the priming solution have also been indicated to improve the germination and the growth of pepper
81   seedlings   [22,23], and other vegetables [24,25].
82   The objective of this study was to evaluate both the response rate of wild seeds of Chili Piquín
83   (*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 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 (ddH$_2$O) 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 H$_2$O$_2$. Seeds were dipped in 0.2 N HCl for 5 min., and rinsed with distilled deionized water (ddH$_2$O) for 2 min. Subsequently were oxidized with 0.5 N hydrogen peroxide for 5 min and newly rinsed with ddH$_2$O 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 °C in darkness for (24, 48 or 72 h) under an aerated solution of (KNO$_3$, K$_2$SO$_4$, NH$_4$NO$_3$, KCl, (NH$_4$)$_2$SO$_4$ or NH$_4$Cl) 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) [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 GA$_3$ treatment [31], was performed using two of the priming treatments [KNO$_{3\,(-15\,atm)}$ and NH$_4$NO$_{3\,(-10\,atm)}$], 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.

126 *2.3. Capsaicinoids determination*:

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

133

134 *2.4. Germination tests:*

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

142 Primary root protrusion to 1 mm was scored as germinated seed. To evaluate root growth, a network
143 of fiberglass of 1 mm^2 was placed under seeds. Primary root length (PRL) was measured in mm.
144 Development germination index (DGI) allows to quantify effects (including FGP and PRL) of
145 treatments $_{(t)}$ respect to control $_{(o)}$ on germination development. DGI was calculated by Zucconi tests
146 [35] by following the formula: $[DGI = 100 \cdot (FGP_{(t)}/FGP_{(o)} \cdot (RL_{(t)}/RL_{(o)})]$ [36].

147 Days to 50% of FGP (T_{50}) and days between 10% and 90% of FGP (G_{10-90}) were also calculated. T_{50} is
148 an inverse measure of mean germination rate, while G_{10-90} is an estimate of germination uniformity
149 (GU) or germination synchrony [37]. To contrast the behavior of treatments $_{(t)}$ to control $_{(o)}$, these
150 parameters were transformed in their respective indices, according to the following formulas: Rate
151 germination index $[RGI = 100 \cdot (T_{50(t)}/T_{50(o)})]$; synchrony germination index $[SGI = 100 \cdot (G_{10-90(t)}/G_{10-90(o)})]$.
152 After germination testing, germinated seeds were transplanted to conventional seedling trays inside
153 a greenhouse to evaluate the number of abnormal seedling generated by each treatment. Abnormal
154 seedling percentage (ASP) and its corresponding abnormality seedling index $[ASI=$
155 $100 \cdot (ASP_{(t)}/ASP_{(o)})]$, were calculated from abnormal plantlets.

156

157 *2.5. Experimental design and statistical analysis:*

158 Treatments were arranged in completely randomized design with four replications of 25 seeds. Data
159 were subjected to multifactorial ANOVA test. Mean separation was performed by Fisher's least
160 significant difference ($LSD_{0.05}$) test if F test was significant at $p < 0.05$ (*).

161 **3. Results**

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

167

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, 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 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_{10-90} 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).

209   Abnormality of plantlets reduced as priming time increases and was lower for -10 atm of Ψ_s. ASI

210   reduced 38% for Cl^-, 62% for SO_4^{2-} and 70% for NO_3^-. Graphic analysis of interactions (data not shown)

211   indicated that 72 h priming treatments with NH_4NO_3 (-15atm) and KNO_3 (-10atm) are optimum regarding

212   the improvement of PGI, MGI, DGI and ASI by adding AG_3 to priming solutions.

213   *3.3. Priming integrated with gibberellic acid treatments.*

214   Average values of germination parameters and indices are presented on Table 3. All treatment

215   significantly reduces capsaicinoids content on primed seeds. Highest CC reduction were obtained on

216   seeds primed with NO_3^- salts and at 200 ppm of AG_3. Combined effects of nitrate priming and AG3

217   reduces initial capsaicinoids contents to 10%. An exponential correlation between CC and DGI were

218   found (data not shown).

219   Pre-sowing with gibberellic acid treatments (Control +100 or +200ppm GA_3) also shows (Fig. 1) a

220   positive effect on germination respective to absolute control for all evaluated parameters (Table 3),

221   except PRL (100 and 200ppm) and G_{10-90} (100ppm).

222   GA_3 significantly reduces latency (FLP) in Piquín chili seeds (Table 3) referred to the absolute control

223   and maintains this effect when it is added to priming solutions (Fig. 1). The addition of GA_3 (at 100

224   or 200 ppm) activates dormant seeds to a rate between 73 and 84% respectively. This latency

225   inhibition causes an increase in PGI of between 30 and 60%. However, GA_3 additions to priming

226   solutions increases FMP respect to their relative to controls.

227   GA_3 significantly increases germination rate (RGI on Table 3) in respect of absolute or relative

228   controls. At 200 ppm this RGI increase by 2.5 times. However, the effect of GA_3 on synchrony is

229   different. While 100ppm has no effect, additions of 200 ppm double the synchrony, reducing intense

230   germination time from 12 to 8 days. These synergic effects of the addition of GA_3 to priming solutions

231   is clearly show for germination percentages on Figure 1. Conversely, 200 ppm GA_3 has no effect on

232   ASI, while 100 ppm GA_3 significantly increases the presence of abnormal seedlings in primed seeds.

233   Gibberellic acid applied alone, significantly reduces the length of the primary root with respect to the

234   absolute control. However, the integrated priming treatment with GA_3, practically duplicate PRL for

235   GA_3 (200 ppm) and increases it by between 50 and 70% for GA_3 (100 ppm). These increases in PRL together

236   with the originated in FGP lead to double or triple values of DGI (associated with GA_3) compared to

237   their respective relative controls. On the other hand, the reduction in PRL (associated with the

238   application of GA_3) regarding the absolute control, neutralizes the positive impact generated on FGP

239   and originates DGI increases, on relative control, like those produced by the halopriming without

240   GA_3.

241   *3.4. Figures, Tables and Schemes*

242   **Table 1**. Average values, ANOVA significance and LSD$_{0.05}$ values of *Capsicum annuum* var. *glabriusculum* seeds

243   and seedling, germinated in darkness at 25 °C following digestion treatments.

	CC ($\mu g \cdot g^{-1}$)	FLP (%)	FMP (%)	FGP (%)	PRL (mm)	DGI	T_{50} (d)	RGI	G_{10-90} (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

244   Capsaicinoids Content (CC; Final Latent Percentage (FLP); Final Mortality Percentage (FMP); Final Germination

245   Percentage (FGP); Primary Root Length (PRL); Development Germination Index (DGI); Days to 50% of FGP

246   (T50); Rate Germination Index (RGI); Days between 10% and 90% of FGP (G_{10-90}); Synchrony Germination Index

247   (SGI); Abnormal Seedling Percentage (ASP); Abnormality Seedling Index (ASI).

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

249   significant difference test.

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

251

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

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

	CC ($\mu g \cdot g^{-1}$)	FLP (%)	FMP (%)	FGP (%)	PRL (mm)	DGI	T_{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$_4$Cl	638c	39.2ab	26.2b	36.5c	17.1abc	421bc	22.4d	113b	21.1f	67a	12.9bc	87bc
(NH$_4$)$_2$SO$_4$	469b	39.9abc	28.0b	32.4b	16.8abc	366bc	22.1cd	115bc	22.1g	64a	11.4b	77b
NH$_4$NO$_3$	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$_2$SO$_4$	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

254   Capsaicinoids Content (CC); Final Latent Percentage (FLP); Final Mortality Percentage (FMP); Final Germination

255   Percentage (FGP); Primary Root Length (PRL); Development Germination Index (DGI); Days to 50% of FGP (T_{50});

256   Rate Germination Index (RGI); Days between 10% and 90% of FGP (G_{10-90}); Synchrony Germination Index (SGI);

257   Abnormal Seedling Percentage (ASP); Abnormality Seedling Index (ASI).

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

259   significant difference test.

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

261

262

263   **Table 3.** Average values, ANOVA significance and $LSD_{0.05}$ values of *Capsicum annuum* var. *glabriusculum* seeds

264   and seedling, germinated in darkness at 25 °C following presowing with gibberellic acid treatments and priming

265   integrated with gibberellic acid treatments.

	CC ($\mu g \cdot g^{-1}$)	FLP (%)	FMP (%)	FGP (%)	PRL (mm)	DGI	T_{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$_4$NO$_3$ (-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
+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

266   Capsaicinoids Content (CC); Final Latent Percentage (FLP); Final Mortality Percentage (FMP); Final Germination

267   Percentage (FGP); Primary Root Length (PRL); Development Germination Index (DGI); Days to 50% of FGP (T_{50});

268   Rate Germination Index (RGI); Days between 10% and 90% of FGP ($G_{10–90}$); Synchrony Germination Index (SGI);

269   Abnormal Seedling Percentage (ASP); Abnormality Seedling Index (ASI).

270   Means within the same column followed by the same letter are not different at p ≤ 0.05 per Fisher's least

271   significant difference (LSD) test.

272   * Significant differences at p ≤ 0.05.

273
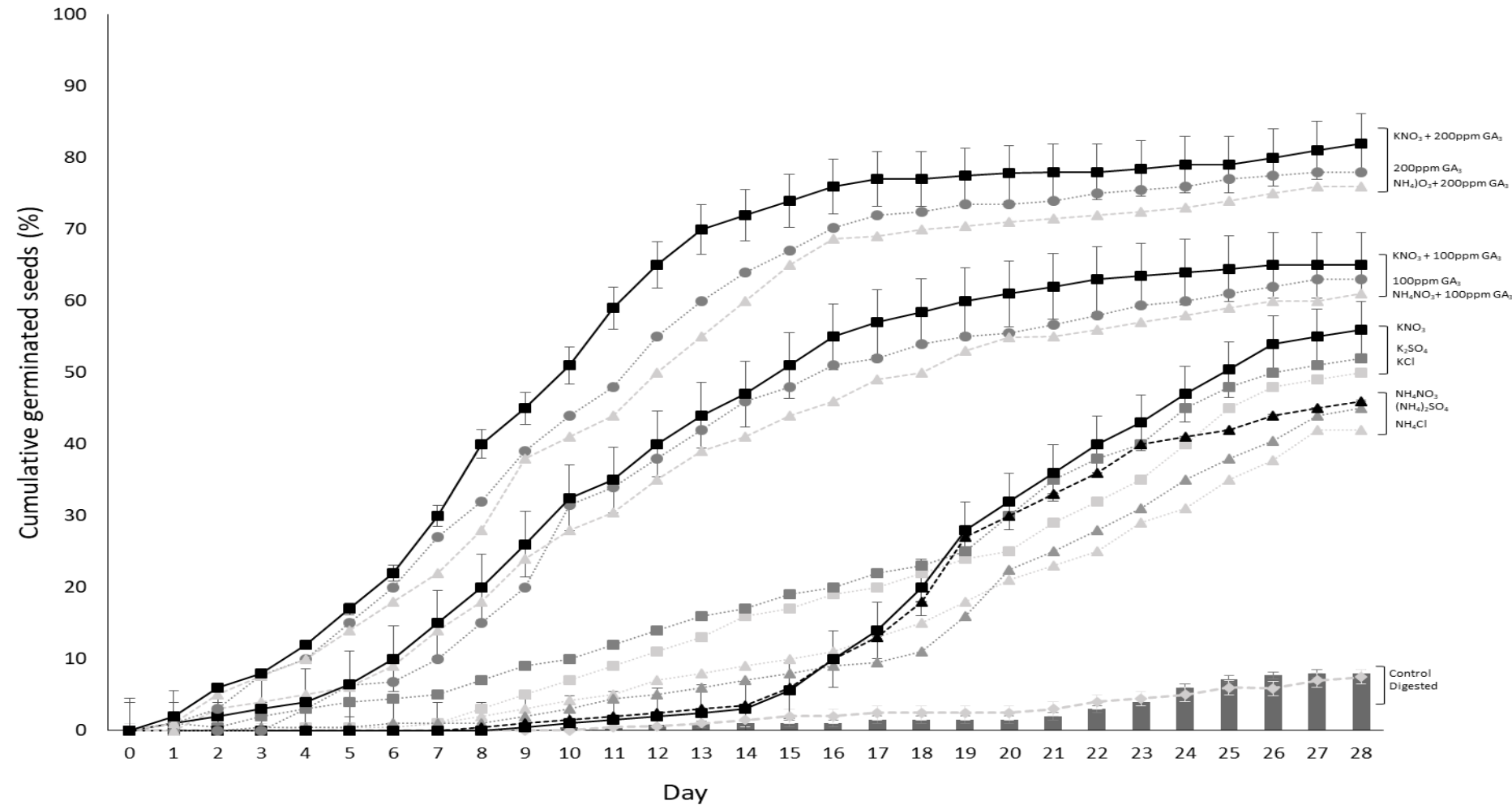
Figure 1. Cumulative germination percentage of Piquín pepper after seeds treatments (digested or primed) monitored for 28 days. Error bars are presented only for control, digested and KNO_3 treatments.

277   **4. Discussion**

278   *4.1. Digestion Treatment.*

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

288

289   *4.2. Priming treatments.*

290   Priming has been proposed as a mechanism of invocation of different stress tolerance of germinating
291   seeds [18]. Seed priming treatments have been applied to various crops under saline conditions
292   [41,42]. Some authors find that a specific ion or salt is not essential in priming pepper seed [43], and
293   other horticultural crop species [15]. Nitrate enhanced germination and seedling establishment rates,
294   under adverse conditions, of onion [44] tomato and asparagus [45], melon [46], watermelon [47], husk
295   tomato [30] and pepper [48,49]. Our results also indicate that nitrate-containing salts are more
296   efficient than nitrate-free salts at promoting germination (except breaking dormancy) of primed
297   seeds. In addition, the effects of priming with KNO_3 seem to be more positive than NO_4NO_3 on main
298   germination and establishment of seedling parameters (except for seed mortality and seedling
299   abnormality). Seed priming stimulates the pre-germination metabolic processes and prepares the
300   seed for primary root protrusion. It increases the antioxidant system activity and the repair of
301   membranes, moreover, the reduction of capsaicinoids on seeds during priming, could contribute to
302   break dormancy and stimulate germinative process on primed seeds. These changes promote seed
303   vigour during germination and emergence [17].
304   Time-course experiments show that effective priming is strongly dependent on both the osmotic
305   potential of the priming solution and the duration of the treatment to avoid "overpriming" [43].
306   Accordingly, the recommended values of osmotic potential to improve the Piquín chili seed
307   germination must be between -10 and -15 atm (-1.0 and -1.5 MPa), the treatment time must be between
308   48 and 72 h.
309   A small number of Piquín pepper studies, very heavily dependent on the origin of seeds accessions
310   and genetic diversity, presented conflicting results [8,26,31,33,50]. Authors do not find positive
311   effects of KNO_3 priming, whereas only see positive effects with GA_3 at extremely high doses (5000
312   ppm). However, none of these studies combine priming with GA_3 at low doses. The undesirable
313   observed effects of seed latency (LGI), mean germination rate (RGI) and synchrony (SGI), could be
314   improved by including gibberellic acid (GA_3) in priming solutions (as shown in Figure 1).

315

316   *4.3. Priming integrated with gibberellic acid treatments.*

317   Halopriming with the addition of plant growth regulators may be an effective way to shorten
318   emergence time and increase stand establishment in watermelon [25] and pepper at low temperatures

319 [37]. Halo-priming using KNO_3 or a growth regulator like GA_3 improves the rate of germination and
320 reduces the mean germination time in endive and chicory [24].

321 The integration of priming with GA_3 was effective in improving germination and establishment of
322 pepper and tomato seeds. Priming, during which germination is suspended, provides an unique way
323 to rapidly and efficiently digest the endosperm by GA-induced enzymes and reduce the mechanical
324 restraints of endosperm thus providing energy to start and sustain embryo growth [51]. Studies of
325 genetics and physiology have shown the important roles of the plant hormones such as abscisic acid
326 and gibberellin in the regulation of seed dormancy and germination [52].

327 Considerable improvements in seed germination performance, an increase in rate and uniformity,
328 and emergence and establishment of seedlings are shown for KNO_3 and GA_3 treatments, in
329 agreement with Tzortzakis [24]. The lowest values of capsaicinoids found on KNO_3 primed seeds
330 together with AG_3 could reduce the allelopathic effect on pepper seed germination. Since high
331 concentrations of capsaicin inhibit the germination of chili seeds [3], the positive effects on
332 germination may be due to the elimination of these as germination inhibitors [9,26]. Finally, our
333 results provide essential information needed for the development of guidelines for the domestication
334 and cultivation of Piquín chili plants.

335 **5. Conclusions**

336 This study showed that it is possible to improve dormancy and germination processes on Piquin chili
337 seeds by priming techniques. Wild Piquin chili seed primed with KNO_3 $_{(-10atm;\ 72h)}$ integrated with GA_3
338 $_{(200ppm)}$ reduced time to germination start (dormancy) and improved germination parameters.
339 Moreover, the results of current study provide essential information needed for the development of
340 guidelines for the domestication and cultivation of Piquín chili plants.
341

342 **Author Contributions:**

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

346 **Conflicts of Interest:** The authors declare no conflict of interest.
347

348 **References**

349 1.   Hayano, C.; Gamez, N.; Medina, L. A. Wild Pepper Capsicum annuum L. var. glabriusculum:
350   Taxonomy, Plant Morphology, Distribution, Genetic Diversity, Genome Sequencing, and Phytochemical
351   Compounds. *Crop Sci.* **2016**, *56*, 1–11, doi:10.2135/cropsci2014.11.0789.

352 2.   Bañuelos, N.; Salido, P. L.; Gardea, A. Etnobotánica del Chiltepín. Pequeño gran señor en la cultura de
353   los sonorenses. *Estud. Soc. Rev. Investig. científica.* **2008**, *16*, 177–206.

354 3.   Derek, W.; Barchenger, D. W.; Bosland, P. W. Exogenous applications of capsaicin inhibits seed
355   germination of Capsicum annuum. *Sci. Hortic. (Amsterdam).* **2016**, *203*, 29–31,
356   doi:10.1016/j.scienta.2016.03.009.

357 4.   Medina-Martínez, T.; Villalón-Mendoza, H.; Pérez Hernández, J. M.; Sánchez-Ramos, G.; Salinas-
358   Hernández, S.; Medina M., T.; Villalón M., H.; Pérez H., J. M.; Sánchez R., G.; Salinas H., S. Avances y
359   perspectivas de investigación del chile piquín en Tamaulipas, México. *Cienc. UAT* **2010**, *4*, 16–21.

360 5.   Kim, H. J. Opportunities and Challenges of Alternative Specialty Crops: The Global Picture. *HortScience*

361   **2016**, *51*, 1316–1319, doi:10.21273/HORTSCI10659-16.

362   6.   Valiente-Banuet, J. I.; Gutierrez-Ochoa, A. Effect of Irrigation Frequency and Shade Levels on Vegetative
363   Growth, Yield, and Fruit Quality of Piquin Pepper (Capsicum annuum L. var. glabriusculum).
364   *Hortscience* **2016**, *51*, 573–579.

365   7.   Demir, I.; Ellis, R. H. Development of pepper (Capsicum annuum) seed quality. *Ann. Appl. Biol.* **1992**,
366   *121*, 385–399, doi:10.1111/j.1744-7348.1992.tb03452.x.

367   8.   Rodriguez-Uribe, L.; Hernandez, L.; Kilcrease, J. P.; Walker, S.; O'Connell, M. A. Capsaicinoid and
368   Carotenoid Composition and Genetic Diversity of Kas I and Ccs in New Mexican Capsicum annuum L.
369   Landraces. *HortScience* **2014**, *49*, 1370–1375.

370   9.   Cano-Vazquez, A.; Lopez-Peralta, M. .; Zavaleta-Mancera, H. A.; Cruz-Huerta, N.; Ramirez R., I.; Gardea
371   B., A.; Gonzalez H., V. A. Variation in seed dormancy among accessions of chile piquin (Capsicum
372   annuum var. glabriusculum). *Bot. Sci.* **2015**, *93*, 175–184.

373   10.   Araiza Lizarde, N.; Araiza Lizarde, E.; Martínez Martinez, J. G. Evaluación de la Germinación y
374   Crecimiento de Plántula de Chiltepín (Capsicum annuum L. var. glabriusculum) en invernadero. *Rev.*
375   *Colomb. Biotecnol.* **2011**, *13*, 170–175.

376   11.   Baskin, C. C.; Baskin, J. M. Germinating Seeds of Wild flowers, an Ecological Perspective. *Perspective*
377   **2004**, *14*, 467–473.

378   12.   Bewley, J. D. Seed Germination and Dormancy. *Plant Cell* **1997**, *9*, 1055–1066, doi:10.1105/tpc.9.7.1055.

379   13.   Paparella, S.; Araujo, S. S.; Rossi, G.; Wijayasinghe, M.; Carbonera, D.; Balestrazzi, A. Seed priming: state
380   of the art and new perspectives. *Plant Cell Rep.* **2015**, *34*, 1281–1293, doi:10.1007/s00299-015-1784-y.

381   14.   Gaius, P. S. Naturalis historia, vol. IV–VII, Books 12–27 (trans: Rackham H, Jones WHS, Eichholz DE).
382   **1949**.

383   15.   Siri, B.; Vichitphan, K.; Kaewnaree, P.; Vichitphan, S.; Klanrit, P. Improvement of quality, membrane
384   integrity and antioxidant systems in sweet pepper (Capsicum annuum Linn.) Seeds affected by
385   osmopriming. *Aust. J. Crop Sci.* **2013**, *7*, 2068–2073.

386   16.   Singh, H. B.; Bisen, K.; Keswani, C.; Mishra, S.; Saxena, A.; Rakshit, A. Unrealized Potential of Seed
387   Biopriming for Versatile Agriculture. In *Nutrient Use Efficiency: From Basics to Advance*; Springer India,
388   **2015**; pp. 193–206 ISBN 9788132221692 | 9788132221685.

389   17.   Ibrahim, E. A. Seed priming to alleviate salinity stress in germinating seeds. *J. Plant Physiol.* **2016**, *192*,
390   38–46, doi:10.1016/j.jplph.2015.12.011.

391   18.   Chen, K.; Arora, R. Priming memory invokes seed stress-tolerance. *Environ. Exp. Bot.* **2013**, *94*, 33–45,
392   doi:10.1016/j.envexpbot.2012.03.005.

393   19.   Zhang, Q.; Rue, K.; Mueller, J. The effect of glycinebetaine priming on seed germination of six turfgrass
394   species under drought, salinity, or temperature stress. *HortScience* **2014**, *49*, 1454–1460.

395   20.   Pandita, V. K.; Anand, A.; Nagarajan, S. Enhancement of seed germination in hot pepper following
396   presowing treatments. *Seed Sci. Technol.* **2007**, *35*, 282–290.

397   21.   Kato-Noguchi, H.; Tanaka, Y. Effects of Capsaicin on Plant Growth. *Biol. Plant.* **2004**, *47*, 157–159,
398   doi:10.1023/A:1027317906839.

399   22.   Albuquerque, K. S.; Guimarães, R. M.; Gomes, L. A.; Vieira, A. R.; Jácome, M. Condicionamento
400   osmótico e giberelina na qualidade fisiológica de sementes de pimentão colhidas em diferentes estádios
401   de maturação. *Rev. Bras. Sementes* **2009**, *31*, 100–109, doi:10.1590/S0101-31222009000400012.

402   23.   García F., A.; Montes H., S.; Rangel L., J. A.; García M., E.; Mendoza E., M. Respuesta fisiológica de la
403   semilla chile piquín [Capsicum annuum var. glabriusculum (Dunal) Heiser & Pickersgill] al ácido

404   giberélico e hidrotermia. *Rev. Mex. ciencias agrícolas* **2010**, *1*, 203–216.

405   24.   Tzortzakis, N. G. Effect of pre-sowing treatment on seed germination and seedling vigour in endive and

406   chicory. *HortiScience* **2009**, *36*, 117–125.

407   25.   Korkmaz, A.; Tiryaki, I.; Nas, M. N.; Ozbay, N. Inclusion of plant growth regulators into priming

408   solution improves low-temperature germination and emergence of watermelon seeds. *Can. J. Plant Sci.*

409   **2004**, *84*, 1161–1165, doi:10.4141/P04-028.

410   26.   Cano-Vazquez, A. Germinación en semilla de chile piquín (Capsicum annuum var. aviculare)., Tesis.

411   Colegio de Postgraduados Montecillo Mx., **2013**.

412   27.   Aloui, H.; Souguir, M.; Hannachi, C. Determination of an optimal priming duration and concentration

413   protocol for pepper seeds (Capsicum annuum L.). *Acta Agric. Slov.* **2014**, *103*, 213–221,

414   doi:10.14720/aas.2014.103.2.6.

415   28.   Schwember, A. R.; Bradford, K. J. Drying rates following priming affect temperature sensitivity of

416   germination and longevity of lettuce seeds. *HortScience* **2005**, *40*, 778–781.

417   29.   Bewley, J.; Black, M. Seeds: physiology of development and germination. *PlenumPress, NewYork, 445p*

418   **1994**.

419   30.   Marín Sánchez., J.; Mejía Contreras, J. A.; Hernández Livera, A.; Peña Lomeli, A.; Carballo Carballo, A.

420   Osmotic Conditioning of Husk Tomato seeds. *Agric. Técnica en México* **2007**, *33*, 115–123.

421   31.   Mireles-Rodriguez, E.; Moctezuma-Balderas, N.; Castro-Nava, S.; Salazar-Hernandez, R.; Lucio-Castillo,

422   H.; Pérez-Jasso, C. Preacondicionamiento en la germinación de cuatro colectas de chile piquín (

423   Capsicum annuum var . aviculare) de Tamaulipas , México. *Acta agrícola y Pecu.* **2015**, *1*, 99–106.

424   32.   Wang-Kyun, R.; Hee-Woong, K.; Geun-Dong, K.; Hae-Ik, R. Rapid determination of capsaicinoids by

425   colorimetric method. *J. Food Drug Anal.* **2017**, *25*, 798–803, doi:10.1016/J.JFDA.2016.11.007.

426   33.   Guerrero, R. Niveles de dormancia en semillas de chiles silvestres de diferentes ecorregiones y desarrollo

427   de protocolos para la germinación y regeneración de accesiones., Tesis; U. A. Aguascalientes, Mx., **2015**.

428   34.   ISTA *Changes to the ISTA Rules for 2016*; Zurich, Switzerland., **2016**; Vol. ISTA Rules;

429   35.   Zucconi, F.; Monaco, A.; Forte, M.; Bertoldi, M. de. Phytotoxins during the stabilization of organic

430   matter. In *Composting of agricultural and other wastes*; Gasser, J. K. R., Ed.; Elsevier: London, **1985**; pp. 73–

431   85.

432   36.   Selim, S. M.; Zayed, M. S.; Atta, H. M. Evaluation of Phytotoxicity of compost during Composting

433   Process. *Nat. Sci.* **2012**, *12*, 69–77.

434   37.   Korkmaz, A. Inclusion of acetyl salicylic acid and methyl jasmonate into the priming solution improves

435   low-temperature germination and emergence of sweet pepper. *HortScience* **2005**, *40*, 197–200.

436   38.   Rodríguez del Bosque, L. A.; Sánchez de la C., R.; SIlva S., M. M. Effect of sunlight regimes on growth

437   and yield of piquin pepper (Capsicum annuum L. var. aviculare). *Rev. Chapingo Ser. Hortícola* **2005**, *11*,

438   357–359.

439   39.   Reid, S.; Armesto, J. J. Avian gut-passage effects on seed germination of shrubland species in

440   Mediterranean central Chile. *Plant Ecol* **2011**, *210*, 1–10, doi:10.1007/s11258-010-9796-8.

441   40.   Jaganathan, G. K.; Yule, K.; Liu, B. On the evolutionary and ecological value of breaking physical

442   dormancy   by   endozoochory.   *Perspect.   Plant   Ecol.   Evol.   Syst.*   **2016**,   *22*,   11–22,

443   doi:10.1016/j.ppees.2016.07.001.

444   41.   Shim, J. S.; Moon, J. C.; Jang, C. S.; Raymer, P.; Kim, W. Effect of potassium nitrate priming on seed

445   germination of seashore paspalum. *HortScience* **2008**, *43*, 2259–2262.

446   42.   Ruttanaruangbowporn, A.; Chanprasert, W.; Tobunluepop, P.; Onwimol, D. Effect of seed priming with

447   different concentrations of potassium nitrate on the pattern of seed imbibition and germination of rice
448   (Oryza sativa L.). *J. Integr. Agric.* **2017**, *16*, 605–613, doi:10.1016/S2095-3119(16)61441-7.

449 43.   Smith, P. T.; Cobb, B. G. Accelerated Germination of Pepper Seed by Priming with Salt Solutions and
450   Water. *HortScience* **1991**, *26*, 417–419.

451 44.   Marín Sanchez, J.; Mejía Contreras, J. A.; Hernández Hernandez., A.; Carballo Carballo.., A.; Peña
452   Lomeli, A. Acondicionamiento osmótico de semillas de cebolla (Allium cepa L.). *Agric. Técnica en México*
453   **2007**, *33*, 63–71.

454 45.   Pill, W. G.; Frett, J. J.; Morneau, D. C. Germination and seedling emergence of primed tomato and
455   asparagus seeds under adverse conditions. *HortScience* **1991**, *26*, 1160–1162.

456 46.   Guzmán, M.; Olave, J. Effects of N-form and saline priming on germination and vegetative growth of
457   galia-type melon (Cucumis melo L. cv. Primal) under salinity. In *Proc. ViIIh Int. Symp. on Protected*
458   *Cultivation in Mild Winter Climates*; **2004**; Vol. 2, pp. 253–260.

459 47.   Loehrlein, M.; Dennis, T. R. Seed Priming of Triploid Watermelon Seed. In *96th Annu. Int. Conf. Amer.*
460   *Soc. Hort. Sci.*; Minneapolis, 27–31 July 1999, **1999**; Vol. 34, p. 481.

461 48.   Garruña H., R.; Latournerie M., L.; Ayala G., O.; Santamaría, J. M.; Pinzón-L., L. Acondicionamiento pre-
462   siembra: Una opción para incrementar la germinación de semillas de chile habanero (Capsicum chinense
463   Jacq.). *Agrociencia* **2014**, *48*, 413–423.

464 49.   Marín Sánchez, J.; Rivas Jacobo, M.; Flores Cano, J.; Rojas Velazquez, A.; Jarquin Gálvez, R. Efecto del
465   priming sobre la calidad fisiológica de semilla criolla de chile ancho (Capsicum annuum L.). *Cienc. y*
466   *Tecnol. Agrop.* **2013**, *1*, 1–6.

467 50.   Cano-Vázquez, A.; López-Peralta, M. C.; Zavaleta-Mancera, H. A.; Cruz-Huerta, N.; Ramírez-Ramírez,
468   I.; Gardea-Béjar, A.; González-Hernández, V. A. Variación en grados de latencia en semillas entre
469   colectas de chile piquín (Capsicum annuum var. glabriusculum). *Bot. Sci.* **2015**, *93*, 175,
470   doi:10.17129/botsci.138.

471 51.   Andreoli, C.; Khan, A. A. Matriconditioning integrated with gibberellic acid to hasten seed germination
472   and improve stand establishment of pepper and tomato. *Pesqui. Agropecu. Bras.* **1999**, *34*, 1953–1958,
473   doi:10.1590/S0100-204X1999001000023.

474 52.   Koornneef, M.; Bentsink, L.; Hilhorst, H. Seed dormancy and germination. *Curr. Opin. Plant Biol.* **2002**,
475   *5*, 33–36, doi:10.1016/S1369-5266(01)00219-9.

476