Colegio de Postgraduados

Variation among populations and effect of gibberellic acid in the germination of wild pepper seeds (*Capsicum annuum* var. *glabriusculum*) from Nortwestern Mexico

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ABSTRACT

Objective: to assess the effects of doses of gibberellic acid (GA) on the germination of seeds of wild chili pepper (*Capsicum annuum* var. *glabriusculum*) to estimate the variation among populations, correlating it to the main climate factors of the seed collection sites in northwestern Mexico.

Design/Methodology/Approach: ripe wild pepper fruits were collected at five sites in the states of Sinaloa and Sonora in northwestern Mexico. After collection, the seeds were immersed in concentrations of 0, 125, 250 and 500 ppm of gibberellic acid (GA). The treated seeds were sown in 60-cavity seedling trays, with Peat moss™ as the substrate.

Results: the application of GA significantly increased germination in all the populations studied. Higher GA doses were more effective in promoting higher germination percentages. Greater weight of seeds was correlated with higher germination percentages. Correlation values were not clearly determinant, however, annual rainfall in the collecting sites of seeds was correlated with the germination percentages of the populations.

Limitations/Implications of the study: wild chili pepper is a species threatened by human activities; by changes in land use, particularly. Also, low germination percentages in this species are limitations for production and conservation.

Findings/Conclusions: the application of GA significantly increased germination in all the populations evaluated, GA doses 250 ppm and 500 ppm were the most effective in promoting germination. Regardless the treatments, variation among populations was high for the germination percentages.

Keywords: germination capacity, gibberellic acid, variation among populations, seed weight to germination capacity correlation.

Citation: Valenzuela-Romero, M. H., Hernández-Verdugo, S., Pacheco-Olvera, A., Osuna-Rodríguez, J. M., López-Orona, C. A., & Osuna-Enciso, T. (2026). Variation among populations and effect of gibberellic acid in the germination of wild pepper seeds (*Capsicum annuum* var. *glabriusculum*) from Nortwestern Mexico. *Agro Productividad*. https://doi.org/10.32854/148f8f34

Academic Editor: Jorge Cadena Iñiguez
Associate Editor: Dra. Lucero del Mar Ruiz Posadas
Guest Editor: Juan Francisco Aguirre Medina

Received: August 21, 2025.
Accepted: March 19, 2026.
Published on-line: April XX, 2026.

Agro Productividad, 19(3). March. 2026. pp: 55-62.

INTRODUCTION

Seed germination is a fundamental process in the establishment and survival of plants under natural conditions. Natural selection is considered to have favored mechanisms that allow seeds to respond to environmental conditions that favor plant development after germination. It is well known that there is variation in germination between populations, among and within individual plants.

The variation in the mechanisms that regulate germination between and within plant species is interpreted as adaptation to the conditions of their habitats at local and regional scales. In addition, the maternal environment (seed origin) is considered to explain the observed differences in germination capacity between populations of the same species.

Under experimental conditions, the application of gibberellic acid (GA) breaks seed dormancy mechanisms, accelerates germination, and frequently replaces the need for environmental stimuli, such as light and temperature in several plant species (Morales-Pizarro *et al.*, 2023), including *Capsicum* (Hernández-Verdugo, 2018; Beltrán-Burboa *et al.*, 2020).

Several studies have reported that seed weight or seed mass vary widely between populations, also among individual plants and within each plant originate physiological changes that are associated with a variety of traits related to adaptation, such as survival, growth, and reproduction (Mendoza-Pedroza *et al.*, 2023). Some researchers reported a positive relationship between seed weight and germination percentage (Gelviz-Gelvez *et al.*, 2020; Mendoza-Pedroza *et al.*, 2023).

Wild relatives of cultivated plants are an important genetic resource that constitutes a primary gene pool, which can help solve problems of current or future agriculture, such as tolerance or resistance to pests and diseases, and increase the quality and quantity of production (Retes-Manjarrez *et al.*, 2016, 2017, 2018; Hernández-Verdugo, 2018; Osuna-Rodríguez *et al.*, 2023). In addition, wild chili pepper fruits are widely consumed in the regions of Mexico where they grow naturally. Previous quantitative studies (Ornelas-Ramírez *et al.*, 2021; Romero-Higareda *et al.*, 2022, 2023) have indicated that wild chili pepper populations in northwestern Mexico maintain high levels of genetic variation between and within their populations.

These populations have also shown high levels of variation between and within their populations in the germination capacity of their seeds. The objective of this study was to assess the effects of doses of gibberellic acid (GA) on the germination of seeds of wild chili pepper (*Capsicum annuum* var. *glabriusculum*) to estimate the variation among populations, correlating it to the main climate factors of the seed collection sites in northwestern Mexico.

MATERIALS AND METHODS

Ripe fruits of wild chili pepper (*Capsicum annuum* var. *glabriusculum*), which afterward were dried in the shade at room temperature for 1 month, the location of collected populations and their geographic and climatic data are shown (Table 1). The populations where we collected seeds were Presa el Oviachic and Mazocahui in the state of Sonora, Yecorato, Lo de Vega and Buyubampo, in the state of Sinaloa (Figure 1).

Germination experiments

The seeds were immersed for 24 h in concentrations of 0, 125, 250 and 500 parts per million (ppm) of gibberellic acid (GA) dissolved in distilled water. The seeds were then put to dry on paper for 24 hours. The treated seeds were sown in 60-cavities seedling trays, using Peat moss™ as substrate. Sixty replicates of seven seeds from each population were sown in each GA treatment.

Table 1. Geographic and climatic data of the collection sites of the populations of wild chili pepper (*Capsicum annuum* var. *glabriusculum*) evaluated.

Population (seed collection site)	Latitude (North)	Longitude (West)	Altitude (m)	Average annual rainfall (mm)	Average annual temperature (°C)
1. Mazocahui	29° 35’	110° 00’	326	586	21.1
2. Presa Oviachic	27° 49’	109° 54’	582	389	25.5
3. Yecorato	26° 26’	108° 12’	405	855	24.1
4. Buyubampo	26° 38’	108° 39’	225	632	25.1
5. Lo de Vega	26° 12’	108° 36’	116	670	23.4

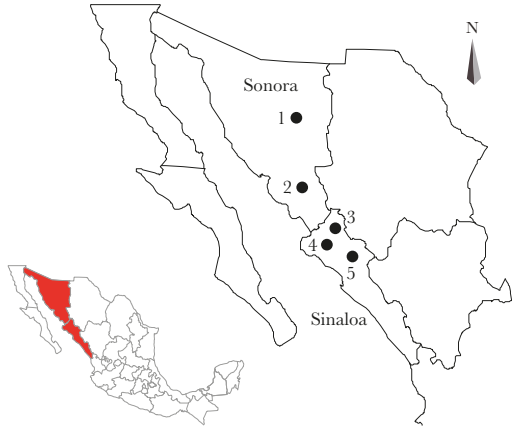

Figure 1. Collection sites of wild chili pepper populations (*Capsicum annuum* var. *glabriusculum*) in the states of Sonora and Sinaloa in northwestern Mexico (names of sites and features in Table 1).

The seeds of each replicate were weighed on a Sartorius™ analytical balance with an accuracy of 0.1 mg. As a germination response variable, the final percentage of germination was recorded at 30 days after planting.

Statistical analysis

In all treatments, the analyzed variable was the germination percentage accumulated at the end of the experiment. The number of seeds germinated daily during the first week was counted, then counted every third day. Count stopped one week after the absence of germination was observed. The germination percentage values were transformed into their arcsine values before the analyses, but the results are reported in percentages. Analyses of variance were performed in two instances. First, a two-ways analysis of variance was performed with all populations with GA treatments as the random factors, to detect general patterns of differences in germination. Univariate (one-way) analyses were then performed between populations for each treatment.

When the differences were significant ($p \leq 0.05$), a multiple mean test (Tukey-Kramer) was performed to determine the differences among the populations studied. Variation in seed weight between populations was estimated using an analysis of variance. Correlation analyses were also performed for germination percentages with the main climate factors of

the collection sites of the populations, and for germination percentages with average seed weight. All analyses were performed using JMP in SAS© version 11.

RESULTS AND DISCUSSION

Gibberellic acid treatments on germination

Overall, germination percentages were very low in all populations non treated with GA, which showed a mean 4.4% of germination within the interval 0.5-6.1% (Table 2, Figure 2A).

In contrast, the application of GA doses 125 ppm, 250 ppm and 500 ppm significantly increased the germination rate in all populations. In those treatments, 250 ppm and 500 ppm doses of GA were the most effective. The average germination percentage of all populations at 250 ppm GA was 31.4%, within the range 2.4-54.2%. The average germination percentage at 500 ppm GA was 30.2% within the 3.8-51.4% range. In the 125

Table 2. Germination percentages (mean±1 SE) of the studied wild chili pepper populations (*Capsicum annuum* var. *glabriusculum*) at different concentrations of gibberellic acid (GA).

Population	Treatments (GA, ppm)				Mean
	0	125	250	500	
1. Mazocahui	6.0	13.3	21.9	12.2	13.4±1.1 c
2. Presa Oviachic	2.3	5.2	2.4	3.8	3.4±0.6 d
3. Yecorato	6.1	39.8	49.1	51.4	36.6±2.0 ab
4. Buyubampo	0.5	56.4	54.2	49.1	40.1±3.3 a
5. Lo de Vega	5.1	27.9	37.39	41.0	27.8±1.7 b
Mean	4.4±0.5 c	26.4±1.7 b	31.4±1.8 a	30.2±1.8 a	

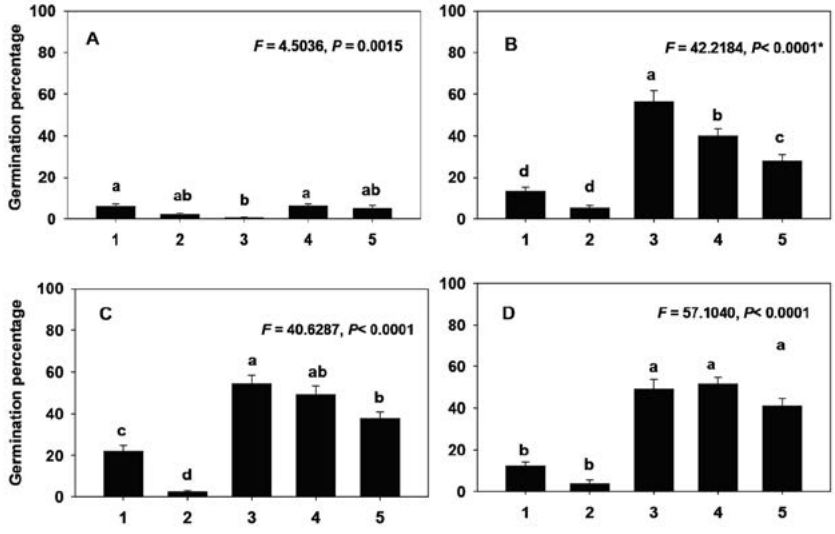

Figure 2. Average final germination percentage (±1 standard error), with A: 0 ppm, B: 125 ppm, C: 250 ppm and D: 500 ppm GA, of the wild chili pepper populations (*Capsicum annuum* var. *glabriusculum*) from Mazocahui, Presa Oviachic, Buyubampo, Yecorato and Lo de Vega (Numbers in X-axis correspond to the populations in Table 1).

ppm GA treatment, the average germination percentage was 26.4% within the rage 5.2-56.4% (Table 3, Figure 3).

The results of this study showed that populations of wild chili pepper (*Capsicum annuum* var. *glabriusculum*) had very low germination capacity in the treatment with no GA (0 ppm), which indicates that these seeds have physiological dormancy that can be broken with the exogenous application of GA (Hernández-Verdugo, 2018).

Of the three concentrations applied, 250 ppm and 500 ppm were the most effective in promoting higher percentages of germination in the seeds of the populations studied. These results are consistent with other studies that have shown that endogenous application of GA is an effective method to increase germination capacity in seeds of other plant species (Morales-Pizarro *et al.*, 2023), including the genus *Capsicum* (Hernández-Verdugo 2018; Beltrán-Burboa *et al.*, 2020).

Table 3. Average seed weight (mean±1 SE) of the populations of wild chili pepper (*Capsicum annuum* var. *glabriusculum*) studied.

Population	Average seed weight (mg)
Yecorato	3.47±0.04
Mazocahui	2.79±0.03
Presa Oviachic	1.81±0.03
Buyubampo	2.94±0.05
Lo de Vega	4.09±0.04

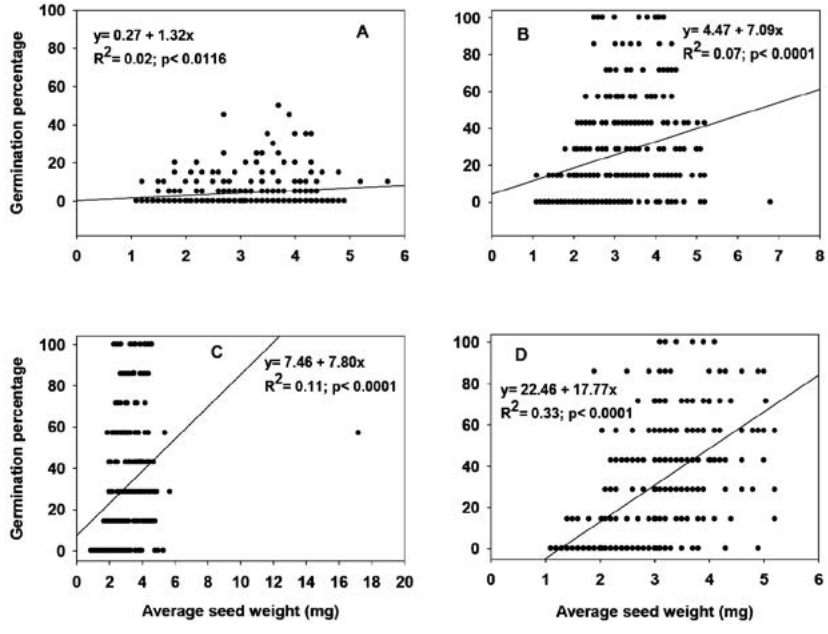

Figure 3. Germination percentage as a function of the average seed weight in treatments with A: 0 ppm, B: 125 ppm, C: 250 ppm and D: 500 ppm GA, of the wild chili pepper populations (*Capsicum annuum* var. *glabriusculum*) from Mazocahui and Presa Oviachic (Sonora), Yecorato, Buyubampo and Lo de Vega (Sinaloa).

Differences between populations in germination

The studied populations of wild *C. annuum* showed significant differences in their germination capacity in all treatments (Table 2, Figure 2).

Considering all treatments, the populations Buyubampo (population 4) and Yecorato (population 3) had the highest germination percentages, with 40.1% and 36.6% as average germination rates (Table 2, Figure 2). The Lo de Vega (population 5) and Mazocahui (population 2) populations showed intermediate germination percentages, while Presa Oviachic population (population 1) presented the lowest average germination percentage of all populations (Table 2, Figure 2).

Variation between populations in seed weight

There were statistically significant differences between populations in average seed weight ($F=545.73$; $p\leq0.0001$). The average seed weight ranged from 1.81 mg in the Presa Oviachic population to 4.09 mg in the Lo de Vega population (Table 3).

Germination variability and climate factors

In regard to germination capacity, all populations differed significantly in all treatments (Table 2, Figure 2). The variation in germination capacity, between populations within the same species, has been interpreted as an adaptation related to habitat characteristics, particularly local climate factors. However, because we observed very few correlations in germination capacity with the main climate factors of the collection sites, we cannot interpret such differences as adaptations to local ecological conditions.

Only the mean annual rainfall showed a positive and marginally significant correlation in the treatments with 250 ppm GA ($R^2=0.686$, $p=0.0845$) and 500 ppm GA ($R^2=0.0710$, $p=0.0731$). Whereas the rest of the climate and geographical variables of the collection sites considered in this study showed no influence on the germination capacity of the wild chili pepper populations studied.

Based on the fact that most rainfall in Northwest Mexico occurs during the germination, growth, flowering and fruiting periods of the studied populations of wild chili pepper, it is likely water availability to be one of the main factors regulating the germination of these populations.

Correlations of seed weight to germination capacity

Four correlation analyses were run between the average seed weight of the five populations studied and the germination percentages. Average seed weight was positively and significantly correlated with germination percentage in each of the four GA treatments (Figure 3), which indicates that heavier seeds are more likely to germinate. These results suggest that the environmental conditions where wild chili-pepper plants grow may influence the weight and germination capacity of their seeds. Other researchers have reported similar results Gelviz-Gelvez *et al.* (2020) and Mendoza-Pedroza *et al.* (2023) showed that larger seeds of Pigeon pea (*Cajanus cajan* (L.) Mill) had higher germination percentages and germination speed rate than smaller seeds.

Seedlings from large seeds had higher dry matter content in roots, stems, and leaves than seedlings from small seeds. Thus, the more vigorous seedlings obtained from large seeds can be more successful during the growth process, which facilitates the absorption of water and nutrients. Gelviz-Gelvez *et al.* (2020) also reported that the heaviest seeds of seven wild plant species growing in semi-arid environments in central Mexico showed significantly higher germination percentage than the smaller seeds ($p \leq 0.05$), which may be due to heavier seeds have a greater capacity to retain water in arid or semi-arid environments.

The seeds of the populations used in this study come from a semi-arid environment, with low rainfall (Table 1) in the states of Sinaloa and Sonora. The high variation observed in seed germination of populations of wild chili pepper in Northwestern Mexico, coincides with the high levels of variation in morphology, genetics, or susceptibility to pathogens and salinity, found in the plants of those populations (Hernández-Verdugo, 2018).

The observed differences in germination patterns among the evaluated populations of wild chili pepper (*Capsicum annuum* var. *glabriusculum*) may be part of a mechanism that allows this species to colonize different habitats. Wild chili pepper populations are distributed from tropical forest sites to highly disturbed localities, such as the vegetation remaining on the roadsides of the low deciduous forest. In addition, this high variation in germination capacity of wild chili pepper may have contributed for reaching the wide geographical distribution this plant currently exhibits in the entire continent of America; this means, from the south of the United States to the south of Peru and north of Argentina (Hernández-Verdugo, 2018).

CONCLUSIONS

Germination of wild pepper seeds from the studied populations was promoted by the endogenous application of gibberellic acid (GA). Of the three concentrations of GA assessed, 500 and 250 ppm were the most effective in promoting higher germination percentages of these seeds. The populations studied differed significantly in their germination percentages in all the treatments tested. Seed germination percentages of the studied populations showed positive but marginally significant correlations with the mean annual rainfall of the collection sites, especially with the 250 ppm and 500 ppm GA treatments. It is possible that the availability of water during the growth and reproduction of wild chili-pepper plants is an important environmental factor for the germination of these seeds. Seed weight was positively correlated with germination percentages in the four GA treatments, indicating that seeds with higher weights showed higher germination percentages than seeds with lower weight.

ACKNOWLEDGEMENTS

To Mexico's National Council of Humanities, Sciences and Technologies (CONAHCYT) for the scholarship granted to Mario Humberto Valenzuela Romero for doctorate studies. To the Autonomous University of Sinaloa, for the support provided to conduct this research.

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