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Effects of temperature wild chili pepper (*Capsicum annuum* var. glabriusculum) germination grown under two light conditions

Efectos de la temperatura en la germinación de chile silvestre (*Capsicum annuum* var. glabriusculum) cultivado bajo dos condiciones de luz

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Abstract

The populations of wild chili pepper (*Capsicum annuum* var. *glabriusculum*) are a genetic valuable resource and the knowledge of the germinative ability, is of great importance for its management and conservation. The effects of the temperature on germination of wild chili grown in two environment of light, and the relation with the weight of 100 seeds of five populations of wild chili of the Northwest of Mexico, were recorded. The mother plants grown in greenhouse conditions with 50 and 100 % of solar light. The treatments applied in the germination cameras matched: Fluctuating temperature (25-35 °C) and constant temperature (25 °C). The treatment variables were the following: percentage of final germination and mean germination time (T_{50}). These variables performed a greatest treatment response of fluctuating temperature. A widely variation among 50 and 100 % of light was recorded with natural conditions. These temperature fluctuations are determinant in germinative processes. The temperature performed a significantly response in terms of the effect in germination velocity (T_{50}) but not in germination percentage. The final germination was not significantly correlated with the seed weight due to maternal effects. In addition, plants grown in greenhouse conditions, differentiate among populations with a genetic basis.

Key words: Final germination, germinative processes, seed weight, T_{50}.

Resumen

Las poblaciones de chile silvestre (*Capsicum annuum* var. glabriusculum) son un recurso genético valioso y le conocimiento de la capacidad germinativa es de gran importancia para su manejo y conservación. Se estimó los efectos de la luz y temperatura sobre la germinación, su relación con el peso de semilla de cinco poblaciones de chile silvestre del Noroeste de México. Las semillas se obtuvieron de plantas que crecieron en invernadero bajo condiciones de crecimiento: 50 y 100 % de luz solar. Los tratamientos de incubación (cámaras de germinación) fueron: temperatura fluctuante (25-35 °C) y constante (25 °C). Las variables fueron porcentaje de germinación final y tiempo medio de germinación (T_{50}). Ambas variables presentaron mayor germinación y velocidad en temperatura fluctuante. Estas fluctuaciones de temperaturas son determinantes en los procesos germinativos. Se observó variación significativa ($P \leq 0.05$) en 50 y 100 % de luz aunque las plantas se adapten al 50 %, tienen mejor adaptabilidad en 100 % de luz. La temperatura tuvo efecto significativo en (T_{50}) pero no en porcentaje de germinación. El peso de semilla no se correlacionó significativamente con la germinación final, esto indica que el ambiente donde crecieron las plantas madres (efectos maternos) tuvo efecto sobre la germinación, además, las plantas hijas crecieron en invernadero, establecen diferencias entre poblaciones con una base genética.

Palabras clave: Germinación final, peso de semilla, procesos germinativos, T_{50}.

Introduction

The study of the germination variability of wild chili pepper populations, known as “Chiltepín”, is a priority task for the management and conservation of this plant genetic resource. However, it is poorly known, little studied, underutilized and it is subject to erosion at an alarming rate.

The habitat fragmentation and destruction where the wild populations of relative plants, are related to the cultivated plants. Consequently, the estimation levels of genetic variation and its pattern distribution among and within populations, are necessary aspects for its management and conservation (Vida, 1994).

Sinaloa and Sonora, are two of the most recognized places by its great natural wild chili populations, commonly known as “Chiltepín”, at the same time became the economic support of the mountain localities and local markets.

The populations of wild *C. annuum* are widely distributed throughout the Mexican Republic of the tropical deciduous forest, pastures, on the shores of the roads and cultivated fields (Hernandez-Verdugo *et al.,* 1999).

Normally it considered the seed size varies and is one of the most important trait in the species or adaptation populations which live in natural conditions due to the influence of germinative ability and in the number and size of the plantlets (Venable, 1992).

In addition, to produce some variation within individuals in the seed weight, which are the number of fertilized eggs and developed within a fruit, phenology and the position within the plant or within the fruit (Obeso, 1993).

Hernandez-Verdugo *et al.* (2010), found high levels of variation in the ability germination of wild relatives of chili populations. However, the amount of variation in germination within the wild populations is poorly unknown. The aim of this research was to study the effects of different amounts of sunlight during the condition of plant growth on the germination of seeds of wild chili, determine the variation among plants in seed germination ability. Additionally, addressed to estimate the effects of temperature on the germination of seeds of Chiltepín and to determine the relationship among seed weight and germination ability.

Material and methods

Development of the experiment

The study was carried out in the greenhouse and laboratory of plant genetic resources of the Agronomy Faculty, Autonomous University of Sinaloa - Mexico, located in the Km 17.5 road to Culiacan- El Dorado. The geographical coordinates of the site are the following: 24° 37’ 29” north latitude and 107° 26’ 36” west. The altitude is 38 m.a.s.l., the wild population geographic data of *C. annuum*, are shown in Table 1.

Table 1. Geographical and climatic data of wild populations of *C. annuum* in northwest Mexico

Id	Population	Latitude (North)	Longitude (West)	Altitude (m.a.s.l.)	PMA (mm)	TMA (°C)
1	Mocuzari	27° 11.810’	109° 07.427’	105	383.7	24.7
2	Yecorato Mezquite	26° 26.349’	108° 12.349’	405	854.6	24.1
3	Yecorato Camino	26° 26.327’	108°12.198’	114	854.6	24.1
4	Lo de Vega	26° 11.596’	108° 36.414’	382	699.5	23.4
5	Texcalama	25° 43.301’	107° 59.184’	296	750.6	25.2

Collections and growth conditions

Ripe fruits of wild chili (*Capsicum annuum* v. *glabriusculum*) of five provenances in northwestern Mexico: Yecorato Mezquite (YME), Yecorato Camino (YCA), Lo De Vega (LDV), and Texcalama (TEX) of Northern Sinaloa State; Mocuzari (MOC) from the South of Sonora, were collected. The collection was carried out in December of 2011. The fruits were transferred to the laboratory in labeled paper bags and stored at room temperature, eight days later, seeds were extracted, counted and weighed. Sowing was established in trays of polystyrene 200 cavities using the substrate of Peat Moss compressed Canadian.

After two months, the seedlings were transplanted into bags for Premium nursery (manufactured from polyethylene with width of 40 and 50 cm height) with 15 kg of barrage soil under growth conditions of 50 and 100 % of the irradiation under the greenhouse roof. The growth condition of 50 % of light solar is obtained throughout a mesh shadow to the 50 % (mesh shade of color black manufactured with Monofilament of polyethylene 100 % Virgin). During the eight months of plant growth (from planting to harvest) was not performed any fertilizer application, the sole irrigation system was dripping irrigation. In the assigned space, plants are rotated randomly every two weeks during the first three months, and consequently every month in the following five months.

Germination experiments

Eight months after the transplant, the mature fruit plants were harvested in the greenhouse. In addition, were saved a month to room temperature and subsequently, 100 seeds per population were extracted for any plant growth condition. The seeds were weighed on an analytical balance (Sartorius Model SARED124S, capability 120 g, Resolution 0.1 mg.). A week after weighing, dry seed were recorded to room temperature and were deposited in four replicates with 25 seeds in petri dishes (6 cm diameter) with bacteriological agar (Bioxon, Made in Mexico) 1 % dissolved in distilled water. The Petri dishes remained in two room temperature conditions, fluctuating (25/35° C) and constant 25° C, with a photoperiod of 12/12 light/darkness hours in germination chambers (Biotronette Lab-Line Imperial III, Model 310, Instruments Incubator., Melrose Park, IL, USA) with light (Status lamp base (2) 360-233-01, Status Lamp Lens (Amber) 360-235-00. Status Lamp Lens (Network) 360-234-00 and Lamp Base 360-233-01) and room temperature. As a response of germination ability, the final percentage and the mean germination time (T_{50}) also known as speed of germination, which consists of the number of days required to germinate the 50 % of the total of sprouted seeds (Hernández-Verdugo, Oyama, & Vázquez-Yanes, 2001), were performed. To obtain this data, germinated seeds are counted every four days after the experiment were started. It was considered as a seed germinated when its radicle measured 1 mm long, approximately.

Statistical analysis

The experimental design for this study was a completely randomized design with respect to the wild chili population. To increase the data normality before the analysis, the values of the percentages germination were transformed into their arcosenos values.

The data were analyzed using nested variance analysis from different variation sources. When the differences among populations were significantly, performed a multiple averages comparison (Tukey test = 0.05).

The data of the final percentages germination were analyzed using a three-way analysis of variance. The sources of variation were plant growth conditions, wild chili populations and incubation at room temperature, were recorded among them. The relation among the seed weight, the germination percentage and speed germination was measured by Pearson correlation. All analyzes were carried out using the JMP SAS ™ statistical package.

Results and discussion

The analysis of variance of three-way showed significant differences in the final percentage of germination, condition of growth and interactions of plant growth condition by incubation temperature, growth condition by population and room temperature incubation by growth condition (Table 2A). The mean germination time, incubation temperature and the interaction among growth condition and wild chili population, also showed significantly differences (Table 2B).

Table 2. Summary of variance analysis with the final percentage germination (**A**) mean germination time (**B**) wild populations of *C. annuum*

Source of variation	DF	SC	F	P
A) % Germination				
Growth condition (C)	1	603.73	7.15	**0.0096**
Incubation temperature(T)	1	156.94	1.86	0.1776
Population (P)	4	615.14	1.82	0.1361
C x T	1	760.94	9.02	**0.0039**
C x P	4	2900.06	8.59	**<.0001**
T x P	4	1310.35	3.88	**0.0072**
C x T x P	4	497.97	1.47	0.2208
Error	60	5060.72		
Total	79	11905.85		
B) T_{50}				
Growth condition (C)	1	784.00	0.70	0.4042
Incubation temperature (T)	1	8083.41	7.27	**0.0091**
Population (P)	4	6782.68	1.52	0.2060
C x T	1	3532.21	3.17	**0.0796**
C x P	4	7790.01	1.75	0.1502
T x P	4	6966.52	1.56	0.1945
C x T x P	4	5133.58	1.15	0.3396
Error	60	66651.93		
Total	79	39072.41		

DF: degrees of freedom; SC: Variation among samples; F: Factor; P: Populations

The mechanisms variation that regulates the ability germination among and within species or populations, are interpreted as an adaptation to the specific conditions of the habitat to local and regional scales (Meyer & Kitchen, 1994; Meyer, Allen, & Beckstead, 1997).

Variation among plant growth conditions

The two growth conditions in the greenhouse caused a differential response in the percentage germination compared to the incubation temperature. In natural growth conditions of 100 % of light, the mean germination percentage was 57.30 %, while in growth conditions of 50 %, the average ability germination was 48.30 %. The light and temperature conditions, are the most important factors that regulates the seed germination in natural conditions. The light is one of the main resources for the plants in their natural environment. The light availability varies temporally and spatially among and within wild populations (Sultan & Bazzaz, 1993).

Variation among incubation temperatures

There were significantly differences only in the meantime germination among incubation temperatures. The seeds required on mean values of 27.33 days to germinate in a constantly temperature of 26.39 days in fluctuating temperature. The percentages variation and germination rates among and within individuals, contributes to reduce the risk subjected to similar conditions (effects) simultaneously during its development, helps to prevent or reduce the competition for resources among plant brothers, increases the seed age distribution in the seed bank, and consequently, raises the genetic variation within a population (Evans & Cabin 1995; Schutz & Rave, 2003).

Variation among populations in growth conditions with 50 % of solar light

There were significantly differences among percentage populations and the mean germination time in the growth condition of 50% of solar light with fluctuating temperature (Figure 1). The populations of Yecorato, Mezquite and Mocuzari performed the highest germination percentages, followed by Texcalama population, Lo de Vega and Yecorato Camino (Figure 1A). In mean germination time, the Yecorato Mezquite population, was occupied more days to germinate, while Lo de Vega and Yecorato Camino populations, germinated in fewest days (Figure 1B). In a constantly room temperature, there were significantly differences among populations in the percentage germination, the mean germination not performed any significantly differences (Figure 2). The Yecorato Camino population, performed the greatest percentage germination with mean values of 56.0 %. On the other hand, the Vega population germinated in lowest percentage with an average of 17.0 % (figure 2A). In the room growth temperature of 50 % solar light, the final percentage and mean germination time were significantly different. The final percentage germination, the fluctuating temperature varied from 55.2% at room temperature and constantly temperature at 41.4%. For a constantly temperature, mean germination time were 57.7 days, the incubation fluctuating room temperature were 24.3 days. The fluctuations temperatures are greatest close to the soil surface and natural cover (Vázquez-Yanes and Orozco-Segovia, 1982). In the sites studied, there are daily fluctuations of similar room temperature and performed a greatest variation used in the present research. Likely, the fluctuating temperature is a key factor in the regulation of seed germination in wild chili populations.

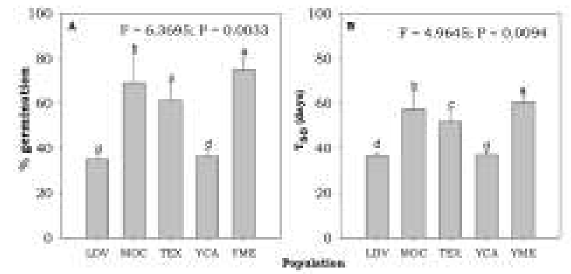

Figure 1. Mean values (± 1 standard error) of the percentage of (A) final germination and mean germination time (B) growth condition of 50% sunlight in fluctuating temperature.

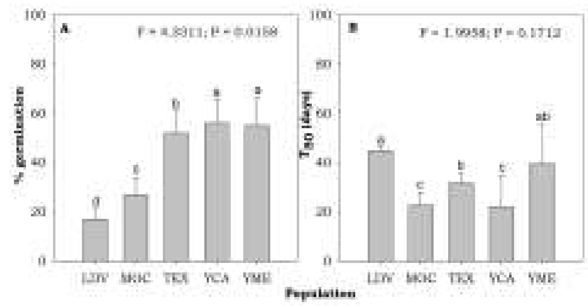

Figure 2. Mean values (± 1 standard error) the percentage of (A) germination and mean germination time (B) growth condition of 50% sunlight at constant temperature.

Variation among populations in a growth condition of 100% solar light

In natural growth conditions with 100 % of sunlight, the incubation temperatures showed not any significantly differences in both variables of germination: at constantly temperature, there were significantly differences in the final percentage germination, the mean germination time not showed any significantly variations among populations (Figure 3). The Yecorato Camino population showed a highest final percentage germination (71.0 %), whereas the Yecorato Mezquite population performed the lowest percentage (42.0 %) (Figure 3A).

In both conditions, growth and incubation temperatures, significantly differences among populations were not performed in percentage and the mean germination time (T_{50}). However, significantly differences among populations in the percentage of final germination at both incubation temperatures of seeds from plants that grew in 50 % of sunlight conditions were observed. There were also significantly differences among populations in the percentage of germination in constantly temperature with seed plants which grew in 100 % of sunlight conditions. In mean germination time, were not significantly differences among populations in fluctuating temperature on seed plants which grew in 50 % of sunlight conditions. These results coincide with those obtained by Hernandez-Verdugo *et al.,* (2010), in seeds collected from plants of these populations in their natural habitat. From this experiment, plants grew up in greenhouse conditions, similar to the natural environment at room temperature, without temperature handle, we can conclude the observed differences in the ability germination among these populations, performed a genetic basis. Other studies have also reported the existence of variation in the ability germination within populations of different plant species (Wulff, 1973; Ayala-Cordero, Terrazas, T., López-Mata, & Trejo*,* 2004).

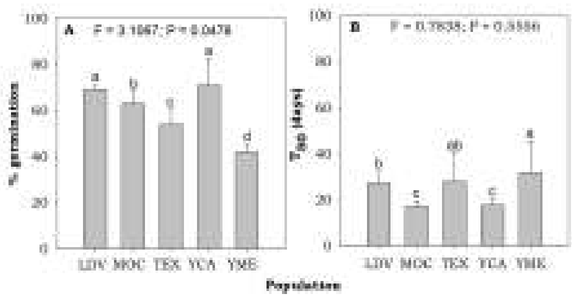

Figure 3. Mean values (± 1 standard error) of the percentage of (A) final germination and mean germination time (B) growth condition of 100% sunlight in constant temperature.

Seed weight variation and its relation to the ability germination

The average seed weight differed significantly among populations and a negative and significantly correlation with lowest values of percentage germination in two temperatures and both growth conditions of 50 and 100 % sunlight (Table 3). This result indicates, the seeds of lowest weight, performs a highly probability to germinate. In the smallest seed populations, will performed a selective advantage over the highest seed weight. In this research, not found any significantly relationship among the germination percentages with the seed weight of plants grew at room temperature of 100 % of sunlight. The differences in relations among seed weight and the ability germination observed in wild chili populations performed an effect on the germination of the evaluated wild chili populations. Seeds germinated in highest percentages and with greatest speed at fluctuating temperature, indicates a great temperature fluctuation and this aspect, estimates to regulate germination of wild chili populations under natural conditions (Hernandez-Verdugo, 2001).

Table 3. Values of the correlation coefficients and levels of significance (P) among the seed weight and germination percentages, mean time germination in two treatments

Treatment and variables	Growth conditions with 50 % solar light		Growth conditions with 100 % solar light	
	r	P	r	P
Fluctuating temperature				
Percentage germination	-0.22	0.34	**-0.42**	**0.05**
Mean germination time	0.07	0.76	-0.05	0.81
Constant temperature				
Percentage germination	0.23	0.32	-0.21	0.35
Mean germination time	0.05	0.82	0.13	0.56

Conclusion

The five populations evaluated performed a variation levels in traits of percentage and mean germination time. Fastest seeds germinated (minor T_{50}) in the treatment of fluctuating temperature and highest percentages compared to constantly temperature, found significantly variation among growth conditions (50% and 100%) of sunlight. However, these plants performed better response in 50% of sunlight. The seed weight not correlated significantly with the germination percentage.

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