

Deterioration of the quality of the piquín pepper seed from four collections in Querétaro and Guanajuato

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Abstract

The objective of the present investigation was to evaluate the quality of piquín pepper seed through the accelerated aging test in piquín pepper (*Capsicum annuum* var. *Aviculare*) collections from the state of Querétaro and Guanajuato. The collections were made in four locations El Patol, El Cuervo and Higuierillas of the municipality of Queretaro and El Tanque of the municipality of Xichu. The vigor of the seed was obtained by the accelerated aging test which consisted of submitting the seeds 24, 48, 72, 96, 120 and 144 h at a temperature of 42 °C and 100% relative humidity, after this period, the seeds are subjected to a normal germination test at 7, 14 and 21 days. The results in the analysis of variance expressed significant effect in genotypes, being the best time for the count performed at 7 days and at 24 h. At 14 days the best times for the witness were zero, 24 h, 48 h and 72 h. On the other hand, the quality of the seed was registered, which presented significant differences for dry stem weight, the highest values were for El Tanque and El Patol. In dry weight of root, weight of one hundred seeds and speed of emergency stand out the collections from El Tanque, El Patol and El Cuervo. Higuierillas presented lower values in the characters indicated above, a difference that could be attributed to environmental conditions.

Keywords: accelerated, aging, collections, germination, vigor.

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Introduction

The piquín pepper, is a wild fruit and is native to Central and South America. From the agricultural point of view, the most important species is *C. annuum*, which is native to Mexico and contains both pepper of large fruits, and pepper of small fruits with a high degree of pungency. The pepper was discovered and introduced to Europe by Columbus and later introduced to the rest of the world (Torres, 2006). The piquín pepper *C. annuum* var. *Avicular*, is recognized as the closest ancestor of the cultivated variety (Pozo *et al.*, 1991), is of natural occurrence and wide distribution in Mexico, when collected, generates significant income during the collection (Montes *et al.*, 2006). Besides being a product of high demand as a condiment and subject to strong anthropogenic pressure, it shows low incorporation of plants to their populations due to the slow and irregular germination of the seed and is at risk a sustained use (Alfonso, 2010). The potential yield of the pepper is in the seed, under the reserve of the management and conditions of the optimum environment (Wall *et al.*, 2002).

The genus *Capsicum* was domesticated in different parts of America. The five domesticated species are *C. annuum* L., *C. baccatum* L., *C. chinense* Jacq., *C. frutescens* L. and *C. pubescens*. It is considered that *C. chinense* is the most variable of the domesticated species in America, is closely related to *C. frutescens* and its distribution in South America is similar (Bosland, 1996).

The piquín pepper is the smallest and the most spicy and in its production period it manages to displace other types of pepper from the market, it is the wild ancestor of *Capsicum annuum*. There is a great diversity of wild peppers or piquín in the country with different shapes, sizes and names according to the region (Del Bosque Rodríguez, 2003).

There are two types of this chili, which has caused much controversy among specialists in the field. The characteristics of the first type show an oval or round chile, and it is found along the roads or rivers in the north of the country, where they call it chiltepín. The other is more elongated, is smaller and has a reputation for being less spicy and is known as piquín pepper. Both are harvested as wild pepper for sale in markets where they reach high prices. The piquín pepper occupies a very important place in the consumption in Mexico due to its rich flavor and smell, besides being widely known, representing a source of income for the rural communities of our country that exploit it (Del Bosque Rodríguez, 2003).

As well as, it is important in the economy of rural families because it is one of the favorite products of Mexicans. In the regions where it is presented, it is an important part of the local economy, mainly during the collection period, generating employment and income for rural communities. In addition, it has been considered in recent years as an important source of food for rural families in our country for what has been established as a productive alternative. The collection and extraction of fruits of wild relatives of this genre has been a way to take advantage of natural resources.

This traditional form of exploitation, in addition to representing additional income to the peasant family economy, is also the cultural expression of a long process of interaction between man and the environment, which is why when proposing alternatives for the use of natural resources we

must consider as much the ecological and economic aspects as the sociocultural ones. That is why it is essential to have a better knowledge of this natural resource and lay technological bases for its use conservation and management with a focus on sustainability. Its domestication and production as a crop is an alternative that helps its conservation and use (Gutiérrez, 2011).

The fruits are very spicy and are often used as a condiment in special dishes in multiple regions of the country. The piquín pepper is an important part of the diet of all Mexicans, is used as a condiment, and is an ingredient of a wide range of typical foods; like sauces, tamales, pozole, chicken broth, menudo, it is also added to drinks, fruits and sweets, among others.

It also has a high nutritional value because it contains a large amount of Vitamin C when consumed as a food, it even contains more than citrus fruits; its vitamin C content reaches 180 mg 100 g⁻¹, although it should not be consumed in excess, it stimulates appetite, increases urination, menstruation and strengthens the stomach. The red pepper can have other important uses to be part of the composition of some drugs used to combat gastrointestinal atony and some cases of diarrhea and the same way is used to fight cancer (Gutiérrez, 2011).

The weight of the seed is an important factor in calculating planting rates. The weight will depend on the size of the seed, its moisture content and the amount of pure seed (Cuevas, 1996). The size of the seed is related to the number of seeds per kilogram and consequently possible number of plants per hectare, also varies by inflorescence, which reflects the differences in times of flowering and nutrition of seeds in development (Wood *et al.*, 1977). Copeland (1976) points out that the availability of water during the development of the seed can intervene in the vigor of the seed indirectly through its influence on the chemical composition of the mature seed.

Germination as the emergence and development of the essential structures that come from the embryo and that show the ability of the seed to produce a normal seedling (Moreno, 1996). According to Delouche (1971), germination is an important and final process in the life of a seed. It represents both the realization and the fulfillment of the basic function of it. Of the great variety of functions that the seed has, the propagation of the genetic inheritance of the plants stands out. It also performs efficiently, as a means of distributing plant populations, through adaptation areas. In addition, it also points out that the last two functions are totally dependent on germination. A seed that has lost its ability to germinate, cannot transmit genetically desirable traits, nor participate in the dispersal of plant populations from one site to another.

Hartman and Kester (1998) determined that the abiotic factors that influence germination are water, oxygen, light and temperature, this last factor being the most relevant in germination, some authors agree that the highest percentage of germination between 97 and 98% are obtained with seeds of fruits harvested from 50 to 55 days after flowering, coinciding with the red pigmentation of the fruit, example of it is the tabasco pepper with a germination of 81% in seeds extracted from fruits harvested in the red state (Lysenko and Butkevich, 1981; Motovani *et al.*, 1981; Edwards and Sunstrom, 1987; Dharmatti and Kulkarni, 1989).

The ISTA (1995) states that the vigor of the seed is the sum of the properties that determine the potential level of activity and behavior of the seed or a batch of seeds, during germination and emergence of the seedling, recommends testing vigor through visual evaluation of seedlings for those species whose seedlings cannot be easily measured by their morphological patterns and because seeds of low vigor produce fusiform plants. This method allows to classify seedlings that have strong and well developed plumules of dark green color, strong primary root, or if it is absent, it must have abundant secondary roots, same, classified as vigorous seedlings.

The physiological quality refers to the viability of the seeds or the potential they have for germination, including vigor, as a result of the expression of factors specific to the genome of the variety (Basra, 1995). Moreno (1996) states that seeds must meet certain quality standards depending on the species to be considered of good physical quality.

Regarding the physical evaluation, Cochran (1974) observed in pepper that the percentage of germination and emergence of large seeds was higher and produced more vigorous, uniform seedlings and with more dry matter compared to small seeds. Edwards and Sundstrom (1987) evaluated the effect of fruit maturity and time of harvest and post-ripening on the germination of Tabasco pepper.

These authors obtained percentages of germination 81% after harvesting red fruits; in addition, the percentage of germination rose 86% after a period of post-maturation of 21 days at 25 °C. Doijobe (1990) studied the effect of the position within the fruit (base, medium and tip) on the quality of pepper seed, this author found that the percentage of germination of the seeds of the base was higher in comparison with those of the middle part and the tip of the fruit. Puente and Bustamante (1991) observed in habanero pepper that as the stage of fruit maturity advanced, there was an increase in the percentage of germination and viability of the seed.

Therefore, the objective of the present work was to evaluate the physiological quality and the deterioration of the seed of four collections of piquín pepper from Querétaro and Guanajuato; through, of the accelerated aging test.

Materials and methods

The research work was carried out in the Laboratory of Seed Analysis of the Technological Institute of Roque, Celaya, Guanajuato, located at km 8 of the Celaya-Juventino Rosas highway, located at 20° 34' 00'' north latitude and 100° 50' 00'' west longitude, at an altitude of 1 765 m.

Four collections of piquín pepper from Querétaro and Guanajuato were used: the community of Patol is located at 20° 47' north latitude and 99° 52' west longitude, at an altitude of 1 847 m, south of the municipal capital of Toliman (INEGI, 2015). The predominant climate is dry of the semi-warm dry subtype, with rains in summer (Bsh); it presents an average annual rainfall of 593.9 mm, and average annual temperature of 20.36 °C (CONAGUA, 2015).

The community of Higuierillas is located at 20° 56' north latitude and 99° 52' west longitude, at an altitude of 1 628 m, north of the municipal seat of Cadereyta (INEGI, 2015). It is characterized by a dry and steppe climate and, according to humidity and temperature, it can be semi-warm or semi-dry. Dry weather is common in winter. The average annual temperature is 17.63 °C and the annual rainfall is 455.44 mm (CONAGUA, 2015).

The community of El Cuervo, is located at 20° 54' north latitude and 99° 55' west longitude, at an altitude of 1 571 meters above sea level, north of the municipal seat of Toliman (INEGI, 2015). The prevailing climate in the middle part of the territory of the municipality, which is the region where the greatest number of inhabitants is concentrated, is dry, of the semi-warm dry subtype, the average annual temperature of 18 °C with average annual precipitation in the westernmost zone of the territory is from 600 to 700 mm (CONAGUA, 2015).

The community of El Tanque is located at 21° 20' north latitude and 100° 5' west longitude, at an altitude of 1 422 m, north of the municipal seat of Xichu (INEGI, 2015). Covered by mountains, with a variable climate, from the warm subtropical to temperate rainfall of 617 mm per year, its annual average temperature is 18 °C (CONAGUA, 2015).

The piquín pepper fruits were collected from wild plants selected at random in each locality, in particular larger fruits. Then they underwent a slow dehydration process, and they were placed in a dry, ventilated place and in the sun.

Once the dehydration of the cover and the placenta of the fruit was carried out, the seed benefit was applied, placed in a square screen of 3 mm diameter; they were manually rubbed to crush them and release the seeds from the placenta. Once crushed, it was placed in a tray with a smaller diameter screen, to eliminate small particles derived from the placentas. Then, they were subjected to the pneumatic separator, in order to eliminate impurities, vain seeds, placenta debris and fruit covers. Then we proceeded to select viable seeds by the method of immersion in water, then proceeded to dry for 24 h, under environmental conditions.

The seeds were classified by weight difference in the gravity table. It is 150 g of chili seeds per sample were used, which were poured into the hopper and by gravity flowed to the conditioned table at 1.5 cm of inclination; likewise, the receiving trays were distributed equidistantly. The samples used allowed the seeds to flow in the trays to be considered.

Weight of 100 seeds. For each collection, four repetitions of 100 seeds were made, to which the weight was recorded with a digital analytical balance (ISTA, 1995).

To promote germination, the commercial product Biogib soluble powder, gibberellic acid (GA3) was used. It 300 seeds were subjected to imbibition for 24 h in a 200 ml bottle of distilled water with Biogib and using only the seeds that were totally immersed and discarding the floating ones. For these tests Petri dishes and absorbent paper were used as a substrate, the seeding was done on paper using four repetitions of 50 seeds. The seeds are disinfected with Captan in a dose of 2 g L⁻¹

of water and placed in Petri dishes. Afterwards, they were placed in a germination chamber at 25 °C, two counts were carried out, the first on the seventh day and the second on day 14, only the normal seedlings were considered (ISTA, 1995; AOAC, 1997).

In this phase vigor (V) and standard germination (G) are determined in two counts, the first seven days after the test is established and the next after 14 days. The records are made in seedlings with root and plumule developed, healthy and without deformations. The data are presented in percentage units.

The vigor of the seed was obtained by the accelerated aging test which consisted of submitting the seeds 24, 48, 72, 96, 120 and 144 h at a temperature of 42 °C and 100% relative humidity, after this period, the seeds are subjected to a normal standard germination test.

Emergency speed. Trays were prepared with soil and substrate composed of vermiculite peat moss, and the seeds were placed in the cavities under a completely random design by three repetitions. Once sown and properly covered, the substrate was watered until saturation was achieved. When starting the coleoptile emergency process, the seedling counts are made daily until the number does not change, the previous allows to obtain the emergency speed following the established by Maguire (1962) making the following formula:

$VE = (\text{number of normal plants/day of the first count}) + \dots + (\text{number of normal plants/day of the final count})$.

Likewise, the dry weight of the seedling (PSP) and the dry radicle weight (PSR) were determined in a sample of 10 seedlings with complete competition, then dried in an oven at 60 °C for 48 h until constant weight. And seedling dry weight. Once the 10 seedlings were obtained at the end of the emergency tray test, they were placed in paper bags and uncovered in an oven at 40 °C for 24 hours, then weighed on a precision analytical balance.

A completely randomized experimental design with a factorial arrangement was used, where the factor (A) are the genotypes and factor (B) are the storage times 0, 24, 48, 72, 96, 120 and 144 h using three repetitions. Comparisons of means were made between treatments, using the minimum significant difference test (DMS).

Results and discussion

In the Table 1 shows the comparison of means for germination through accelerated aging. According to Taiz and Zeiger (2010) mention that the germination process is activated once the seed tissues are hydrated, which generates a chain of irreversible events, where the process begins with the degradation of sugars in the endosperm, as well as the import of these into the embryo to promote growth; also, genes involved in germination are activated.

Table 1. Comparison of means for standard germination in collections of piquín pepper from the state of Queretaro and Guanajuato. Roque, Celaya, Guanajuato 2015.

Genotype	Germination (%)		
	7 days	14 days	21 days
Higuerillas	6.57 a	24.45 a	36.1 a
El Tanque	0.97 b	11.05 b	30.1 b
El Patol	3.04 b	24.79 a	38.7 a
El Cuervo	5.66 a	24.81 a	39.38 a
DMS (0.05)	2.28	5.29	5.05
\bar{X}	4.06	21.28	36.07
Aging accelerated (h)			
0	3.38 c	26.6 a	36.37 a
24	11.87 a	28.49 a	33.28 a
48	7.69 b	29.33 a	36.77 a
72	3.38 c	26.59 a	36.04 a
96	2.03 c	17.84 b	34.6 a
120	0 c	6.23 c	39.13 a
144	0 c	13.84b	36.27 a
DMS (0.05)	3.49	8.09	7.71
\bar{X}	4.05	22.43	36.06

Equal letters mean there are no significant differences.

When carrying out the germination counts at 7 days, two groups were presented where the first group was formed by the genotype from Higuerillas and El Cuervo who obtained the highest percentage of germination with 6.57 and 5.66%, the second group was made up of the genotypes of El Patol with 3.04% and well below the average, the El Tanque genotype was positioned with 0.97%. On the other hand, for the counts at 14 days the best genotypes were Higuerillas, El Patol and El Cuervo with 24.45, 29.79 and 24.81%, respectively; however, the seed was again evaluated for El Tanque with 11.05% who presented the lowest value. Finally, the count performed at 21 days, with the exception of El Tanque with 30.1% of normal seedlings, the genotypes of Higuerillas, El Patol and El Cuervo had higher ranks of 36.1 to 39.38%. It should be noted that the genotype most affected by temperature and high relative humidity (42 °C and 100% HR) was the collection of the tank in all the germination samples, which indicates that this material has susceptibility to accelerated deterioration and therefore low longevity.

The best time for the count performed at 7 days was 24 hours after deterioration. This coincides with that described by Dell'Aquila (2004) who mentions that hydration occurs in the first 8 h of imbibition, in seeds of chili (*Capsicum annuum* L.) and tomato (*Lycopersicon esculentum* L. and *Solanum lycopersicum* L.). This behavior contrasts with that observed in corn seeds (*Zea mays* L.) by Cruz-Perez *et al.* (2003), in which the endosperm presents slow imbibition in the first 10 h. On the other hand, at 120 and 144 h of aging, it turned out to be counterproductive when no germinated seeds were present; that is, as time goes on exposure to stress the seed loses its viability.

Regarding the counts performed at 14 days, the best times were presented: witness zero (26.6%), 24 h (28.4%), 48 h (29.33%) and 72 h (26.59%). On the other hand, all genotypes presented a negative response after 96 h of aging; that is, the number of normal seedlings decreased.

On the other hand, in the evaluation performed at 21 days, all evaluated aging times did not show statistical differences; that is, they showed very similar results. Regarding this, Doijobe (1990), in the study of vigor and viability of pepper, we found all the statistically similar counts that the seeds extracted from fruits collected in mature state have a greater germination capacity and seedling vigor, however, the Fruits can be harvested at an earlier stage without affecting their quality. On the other hand, Almanza (1993), in bromatological studies carried out in piquín pepper found that for its sowing it is necessary to have quality seeds, which is obtained from fully ripe (red) fruits of healthy plants. If it is extracted from green fruits and matured later, the seeds will present problems in their germination or will produce weak seedlings with poor development.

On the other hand, Bañuelos *et al.* (2008); Araiza *et al.* (2011) reported low germination rates in piquín pepper seeds, which has been attributed to the impermeability and hardness of the seminal cover, to the low permeability of the endosperm and to a deep latency of the embryo. These results, independently of the treatment with gibberellins, show that the best evaluation time is at 21 days since this time the highest germination percentages were presented at 21 days, according to the results of the means (Table 1).

Del Bosque Rodríguez *et al.* (2004) mention that less than 5% germination has been registered during the first month of planting seeds of wild populations of chili pepper, this is because the seed presents latency, since the seed coat is composed of epicuticular wax and an outer layer hard that make it waterproof, which influences an adequate absorption of water. These results agree with those of this experiment at 7 days of evaluation, presenting an average of all the genotypes of 4.06% of germination and through the times of accelerated aging found an average of 4.73%. According to Meyer (1994), the impermeability of the seed coat can cause dormancy in the seed. However, given the individual characteristics of the seeds of the same species, germination is not a process that is expressed simultaneously in the same population (Bewley, 1997).

In the case of the piquín pepper family, *Solanaceae*, it has been documented that it presents non-deep physiological dormancy, in which the germination is influenced by factors such as phytochromes, temperature and regulators of plant growth such as abscisic acid and gibberilins (Baskin and Baskin, 2004).

In recent years, new techniques have been studied to improve germination; these include the use of physiological hydration-dehydration treatments, which were found to be adequate to increase, accelerate and synchronize the germination of fresh and aged seeds of many crops (McDonald, 2000, Sanchez, 2005) and at the same time allow the exchange of water and gases through the hard and cutinized covers.

Some researchers found that in seeds collected at higher elevations, within the range of distribution of the species, the germination percentages were higher, because the percentage of germination and the initial growth can be influenced by the altitude in which it was collected seed (Bolaños and

Vera, 1997). According to our results, the genotype with less germination potential in all the samplings was the collection of El Tanque from the state of Guanajuato located at 1 422 meters above sea level. The other three locations where the collections were made have lower altitude from (1 571 to 1 847 m) of the state of Querétaro and all have a higher percentage of germination; which indicates that this characteristic is affected by altitude.

Other studies show that the growth of plants at lower elevations is higher, which results in a higher percentage of establishment of the seedlings, so that at higher altitude the growth of the seedlings is less germination (Viveros-Viveros, 2009).

In Table 2, the comparison of means test is shown. In which it can be observed that dry stem weight the highest values were for El Tanque and El Patol with 0.296 and 0.27 g, respectively. In root dry weight (PSR), weight of one hundred seeds (P100s) and emergence speed (VE), the collections from El Tanque, El Patol and El Cuervo stand out with the best behavior; while the pique chin genotype located in Higuierillas had the lowest values in the abovementioned characters. This difference was eight times less than the collection of El Tanque. The collection of castor oil plant showed the lowest weight of seed, this last variable does affect vigor and germination frequently (Cubero, 1999).

Table 2. Comparison of means of initial stem vigor and seed weight in piquín pepper collections. Roque, Celaya, Guanajuato 2015.

Genotype	PSV	PSR	P100S	VE
		(g)		(num.)
El Tanque	0.296 a	0.0410 a	0.316 a	32.729 a
El Patol	0.27 a	0.0334 a	0.31 a	29.204 a
El Cuervo	0.251 b	0.028 a	0.304 a	26.501 a
Higuierillas	0.243 b	0.0243 b	0.2629 b	4.978 b
DMS (0.05)	0.042	0.014	0.026	9.928

Equal letters mean there are no significant differences. PSP= stem dry weight, PSR= root dry weight; P100S= weight of one hundred seeds; VE= emergency speed.

The seed from El Tanque showed a better response than that obtained from El Patol, a difference that could be attributed to environmental conditions. The community of El Tanque is located at 1 422 m altitude, records a rainfall of 617 mm and an average annual temperature of 18 °C. While the Patol is located at 1 847 m in height, 593.9 mm in precipitation and 20.36 °C. Another factor that could be attributed is that the plants where the piquín pepper fruits were collected in El Tanque coexist with a vegetation covered by mountains and variable climate. This condition could be attributed to the genotype environment interaction (Cubero, 1999). It should be noted that the collection of El Patol was the one with the lowest percent germination, which indicates a negative correlation, which is considered irregular.

In this sense, Geneve and Kaster (2001) in a study of size and growth of seeds, mention that the growth of seedlings is useful to measure vigor but the distribution of weight between the aerial part and radical of the seedling would represent the maternal condition of growth of the seed in a new

environment. Alderete *et al.* (2005) concludes that the development of the seedling and its vigor, maintains a close relationship according to the origin and environment where it is sought to propagate its crop.

Conclusions

There was a strong phenotypic variation for all the registered characters, due to the intra and interpopulation genetic diversity of the collections. The highest germination rate was obtained after 21 days of evaluation of the test. By increasing the exposure time of the seeds at high temperatures and 100% relative humidity (deterioration), obtaining normal seedlings decreased significantly after 14 days of evaluation. With respect to the initial vigor of the seedling, the genotype collected in El Tanque presented the best behavior; in dry weight of stem and root 0.29 and 0.041 g, weight of 100 in seeds 0.316 g and in emergency speed. Finally, the characteristics of the proximal analysis were not related to the initial vigor of the seedling, seed weight and seed vigor. However, they had a negative relationship at 7 and 14 days of the test.

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