Article

Age, benefit and gibberellic acid affect the germination and production of piquín pepper plant

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Abstract

To obtain pepper or wild pepper seeds, the way of collecting or producing, the extraction technique, the selection, the packing, the form and time of storage and the conditioning prior to sowing, affect the germination and viability of the seed and the production of seedlings. The above, added to the natural latency, represent an important obstacle for the domestication and commercial production of this species. Therefore, the objective of this study was to evaluate germination and seedling production in relation to age, benefit and conditioning prior to sowing. Seeds of fresh red fruits of an ecotype of the region of Linares, Nuevo León, Mexico of 0, 2, 4 and 12 months of age after extraction were used. The benefit consisted of drying the seed with forced air up to 9% humidity, standardize by weight, pack in aluminized polyethylene bag and hermetically sealed and store at 15 °C. The conditioning was immersed with gibberellic acid at 5 000 mgL^{-1} for 12 h before planting. Germination, hydration rate, tetrazolium staining and seedling production were evaluated. The results show that the newly harvested seeds do not germinate due to immaturity of the embryo; after two months of rest the seed germinates, the benefit maintains its viability for more than a year, conditioning with gibberellic acid prior to sowing, increases the percentage of germination and the production of seedlings.

Keywords: *Capsicum*, wild peppers, seeds.

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Introduction

The demand for “piquín” or “del monte” wild peppers (*Capsicum annuum* var., *aviculare*, Dierb D'Arcy and Eshbaugh or *glabriusculum*) is on the increase, as traditional selling of fresh and dried fruit on the street and markets regional, the commercialization of derivative products such as pickles, sauces and dehydrated in commercial stores chains is added. There is no evidence of commercial production, only its backyard cultivation for self-consumption (Latournerie *et al*., 2002; Pedraza and Gómez, 2008). Therefore, this market is supplied almost entirely by the collection of wild fruits (Medina *et al*., 2002; Bran *et al.*, 2007).

This situation has made the collection of this species more intense and aggressive, because collectors that, in their eagerness to harvest a greater quantity, do not collect only the fruits but cut the productive branches and even the whole plant, limiting their possibilities of regeneration. This has caused the disappearance of the species in some regions, especially those close to population centers. If this situation continues, it is put at risk if not the species, if important ecotypes (Bañuelos *et al.*, 2008; Araiza-Lizarde *et al.*, 2008). Therefore, it is necessary to look for options to conserve the existing wild populations and it is considered that one of the strategies is to discourage the collection of fruits of wild plants, through the domestication and agronomic production of these peppers, using the technology that is currently used they produce varieties of commercial peppers. However, for the establishment of these production programs, the problem of germination of the seed must first be solved, which has germination rates ranging from 5 to 80% (Ramírez-Meraz, 2001; Rodríguez Del Bosque *et al*., 2003).

The low and variable germination rate is an important impediment to having certified seed that allows to establish lots of commercial production of this species. The percentage of reduced germination in the piquin pepper seed is attributed to physical or physiological latency, caused by the impermeability of its seed coat or cover, immaturity of the seed or resting of the embryo (Besnier, 1989, Ramírez-Meraz, 2001; Rodríguez Del Bosque *et al*., 2003). To solve this problem, different techniques are reported, including chemical and physical scarification, conditioning or seed treatment prior to sowing with gibberellic acid, potassium nitrate, hydrogen peroxide (Rodríguez Del Bosque *et al.*, 2003; De la Rosa *et al*., 2012; Cano-Vázquez *et al.*, 2015).

However, the results are very variable and low germination rates persist. On the other hand, previous studies show that the age of the seed significantly affects its germination, in addition there is no evidence that the seeds of the wild peppers are benefited and stored properly. The objective of this work was to evaluate seed germination and seedling production, depending on the time elapsed from the extraction to the sowing, the benefit to the seed and the conditioning of the seed with gibberellic acid prior to sowing.

Materials and methods

The work was carried out in the Seed Production and Testing Laboratories of the Seed Technology Training and Development Center (CCDTS) and in the Horticulture Department of the Antonio Narro Autonomous Agrarian University, in Buenavista Saltillo, Coahuila, Mexico, during the period from 2006 to 2011. The plant material consisted of seeds of different ages, 0, 2, 4 and 12 months, obtained from fresh red fruits from collections of the Linares region, Nuevo Leon, Mexico.

Extraction and benefit of the seed.

The seed was extracted by macerating the fruits with hands and latex gloves, the macerate was placed in drinking water and the seeds were allowed to settle, then dried at room temperature. Half of the seed was conditioned and the other half was only extracted, dried by sun exposure and stored in a conventional way in a Ziploc brand polyethylene bag and at the ambient temperature of the seed laboratory of the CDDTS. The conditioning consisted of drying the seed with forced air, until reaching a humidity of 9%, for which a South Dakota brand blower was used, it was homogenized by weight and size and it was stored in Uline brand plastic bags of 10 × 20 cm, hermetically sealed and at a temperature of 15 °C (Aguirre and Peske, 1998; Reveles-Hernández *et al.*, 2013).

Germination tests

Four ages of the seed, 0, 2, 4 and 12 months after extraction were evaluated, seeds benefited and not benefited and seeds preconditioned with gibberellic acid at 5 000 mgL^{-1} and seeds without preconditioning (dry seeds) each treatment with three repetitions and each repetition with 50 seeds. The experimental design was factorial A × B × C, where: A= age, B= benefit and C= conditioning (Zar, 1996). The seeds were germinated in Petri dishes on Whatman No. 1 filter paper in a Lab-line branching chamber at 25 ±1 °C, with 8 hours of light and 16 hours of darkness. Germinated seeds, ungerminated seeds and abnormal plants were recorded (AOSA, 1993).

Imbibition test of the seed

This test was performed additionally to determine if the seed was impervious to water. The 50 seeds of each age were weighed with four repetitions on an Ohaus brand precision balance, model Adventurer Pro, then placed in a 13 × 100 mm test tube, 10 cc of distilled water was added with an Eppendorf brand micropipette and by difference of the volume, the water absorbed was quantified, the excess water was removed and the hydrated seed was weighed again, to determine the weight increase by the water absorption. This test was performed at 12, 24, 36, 48 and 60 h.

Viability test with tetrazolium

To determine the viability of the seed, the 2, 3, 5-Triphenyl test, Tetrazolium chloride (TZ), Sigma-Aldrich brand (ISTA, 2004) was carried out. It started with an osmoconditioning of the seed, which consists of placing the seeds in distilled water for 16 hours. Once the seed was hydrated, longitudinal cuts were made with a scalpel, to expose the cotyledons. The sections were placed in a test tube of 13 × 100 mm, adding the solution of 2, 3, 5 Tetrazolium Trifenil chloride 0.1% to cover the seed, then the test tubes were wrapped with aluminum foil to avoid light and they were placed in a Lab-Line brand incubation chamber at 30-35 °C for 90 min. Subsequently, in a Carl Zeiss brand stereoscope, seed staining was observed.

Seedling production

In this test, three different ages of the seed, 2, 4 and 12 months were evaluated (the seeds were removed recently harvested, because there was no germination in that test), seeds benefited and not benefited, seeds preconditioned with gibberellic acid at 5 000 mgL^{-1} and seeds without preconditioning (dry seeds); each treatment with three repetitions and each repetition with 50 seeds. The experimental design was factorial A × B × C where, A= age, B= benefit and C= conditioning (Zar, 1996). The seeds were planted in polystyrene trays of 200 cavities and as a substrate moss peat moss brand Sunshine was used. At 48 days after sowing, percentage of seedlings suitable for transplanting, height and diameter of the seedlings and number of true leaves were evaluated.

Results and discussion

Germination tests

The results show that age and benefit, are the factors that have the most effect on germination, the conditioning with gibberellic acid at 5 000 mgL^{-1}, was also significant, but to a lesser degree. The highest germination percentages of piquín or wild pepper were obtained with the seeds of 2 and 4 months of age, which were benefited and conditioned before sowing with gibberellic acid (Table 1). The piquín or wild pepper seed that is currently used for research, backyard production or small scale, comes from collections that are made directly in the field, fruits left behind vendors or nuts that retain fans of this crop or that are purchased in stores.

The form of storage is also very varied and goes from the conservation in nuts or the extraction and conservation in containers, between the most common, polyethylene bags, paper and glass jars. The management of the seed, from the production or collection, form of extraction, selection, conditioning, drying and storage, as a whole is called profit (Aguirre and Peske, 1988). This benefit has a high impact on the germination and viability of the seed and no evidence was found to be made on piquin pepper seeds. This may be a cause of the low and variable germination rate reported from 5 to 80% (Ramírez-Meraz, 2001; Rodríguez *et al*., 2003; Cano-Vázquez *et al*., 2015).

Among other possible causes, are cited from myths such as the fact that the seed of wild pepper, require passing through the digestive system of birds to germinate (Ariza-Lizarde *et al*., 2011). The investigations to determine the causes of the latency, have basically started from two hypotheses; One is that the seed has physiological latency, for this purpose germination tests have been carried out with hydrogen peroxide (Flores *et al*., 2008), potassium nitrate and gibberellic acid (García-Federico *et al*., 2010; Araiza-Lizarde *et al*., 2011), another hypothesis is that the seed presents physical latency, attributed to the impermeability of the testa or seminal cover and to the low permeability of the endosperm (Bañuelos *et al*., 2008; Araiza *et al*., 2011), for this studies of scarification, stratification and previous hydration of the seed are reported. Therefore, in a complementary way, this study included a conditioning test with gibberellic acid and another one of imbibition that are cited as the most common tests to check whether there is physical or physiological latency.

Table 1. Effect of age, benefit, gibberellic acid and interactions on piquín pepper germination.

Treatments	Mean	Standard error
Age 0 months	0.5 c	0.35
Age 2 months	74.5 ab	5.22
Age 4 months	90.66 a	2.26
Age 12 months	42.5 bc	12.45
With benefit	37.25 b	7.99
Without benefit	66.83 b	8.06
Without conditioning with gibberellic acid 5 000 mg L^{-1}	49.91 b	8.48
With conditioning with gibberellic acid 5 000 mg L^{-1}	54.16 b	8.69
Age 0 ∗ without benefit	0.33 c	0.33
Age 0 ∗ with benefit	0.66 c	0.66
Age 2 ∗ without benefit	61 b	6.08
Age 2 ∗ with benefit	88 ab	3.18
Age 4 ∗ without benefit	86.33 ab	3.63
Age 4 ∗ with benefit	95 a	1.34
Age 12 * without benefit	1.33 c	0.98
Age 12 ∗ with benefit	83.66 ab	1.81
Age 0 ∗ without conditioning	0 c	0
Age 0 ∗ with conditioning t	1 c	0.68
Age 2 ∗ without conditioning	67.33 b	8.02
Age 2 ∗ with conditioning	81.66 ab	5.91
Age 4 ∗ without conditioning	89.66 ab	2.8
Age 4 ∗ with conditioning	91.66 a	3.77
Age 12 ∗ without conditioning	42.66 bc	19.12
Age 12 ∗ with conditioning	42.33 bc	17.78
Without benefit ∗ without conditioning	34.33 bc	11.14
Without benefit ∗ with conditioning	40.16 bc	11.9
with benefit ∗ without conditioning	65.5 b	11.51
with benefit ∗ with conditioning	68.16 b	11.78
Age 0 ∗ without benefit ∗ without conditioning	0 c	0
Age 0 ∗ without benefit ∗ with conditioning	0.66 c	0.66
Age 0 ∗ with benefit ∗ without conditioning	0 c	0
Age 0 ∗ with benefit ∗ with conditioning	1.33 c	1.33
Age 2 ∗ without benefit ∗ without conditioning	50.66 b	4.37
Age 2 ∗ without benefit ∗ with conditioning	71.33 ab	7.68
Age 2 ∗ with benefit ∗ without conditioning	84 ab	5.03
Age 2 ∗ with benefit * with conditioning	92 a	3.05
Age 4 ∗ without benefit ∗ without conditioning	86.66 ab	5.45
Age 4 ∗ without benefit ∗ with conditioning	86 ab	6
Age 4 ∗ with benefit ∗ without conditioning	92.66 a	0.66
Age 4 ∗ with benefit ∗ with conditioning	97.33 a	1.76
Age 12 ∗ without benefit ∗ without conditioning	0 c	0
Age 12 ∗ without benefit ∗ with conditioning	2.66 c	1.76
Age 12 ∗ with benefit ∗ without conditioning	85.33 ab	2.9
Age 12 ∗ with benefit ∗ with conditioning	82 ab	2.3

Means with different letters are statistically different (Tukey $p \leq 0.05$).

Imbibition test

The results show that the piquín pepper seed absorbed a greater volume of water during the first 12 hours, while in the subsequent 48 h the absorption was minimal. The above shows that the seed is not impervious to water. The impermeability of the testa or seminal cover is reported as the most probable cause of the physical latency of the piquín pepper seed (Ramírez-Meraz, 2001, Rodríguez-Del Bosque *et al*., 2004). However, the imbibition tests show that the seed is hydrated during the first 12 hours of exposure to water (Figure 1), and therefore the latency is not associated with the impermeability of seed coat or cover (García-Federico *et al.*, 2010; Prado-Urbina *et al*., 2015).

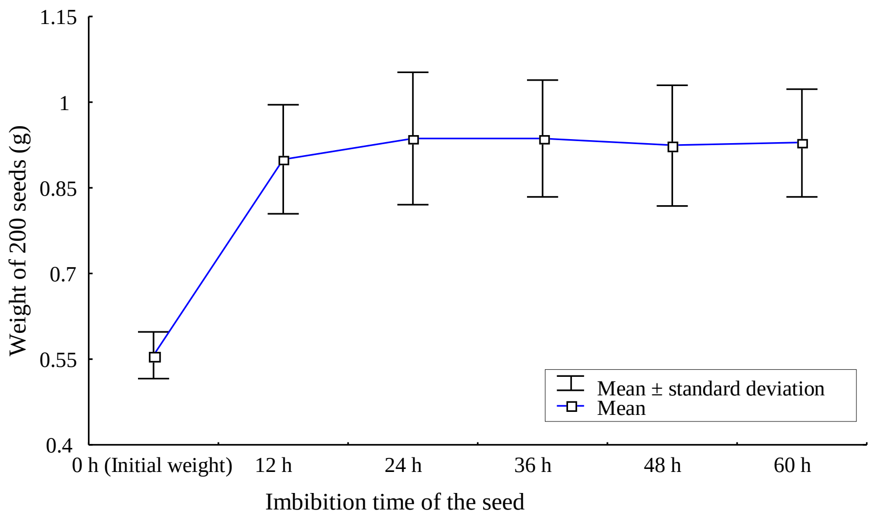


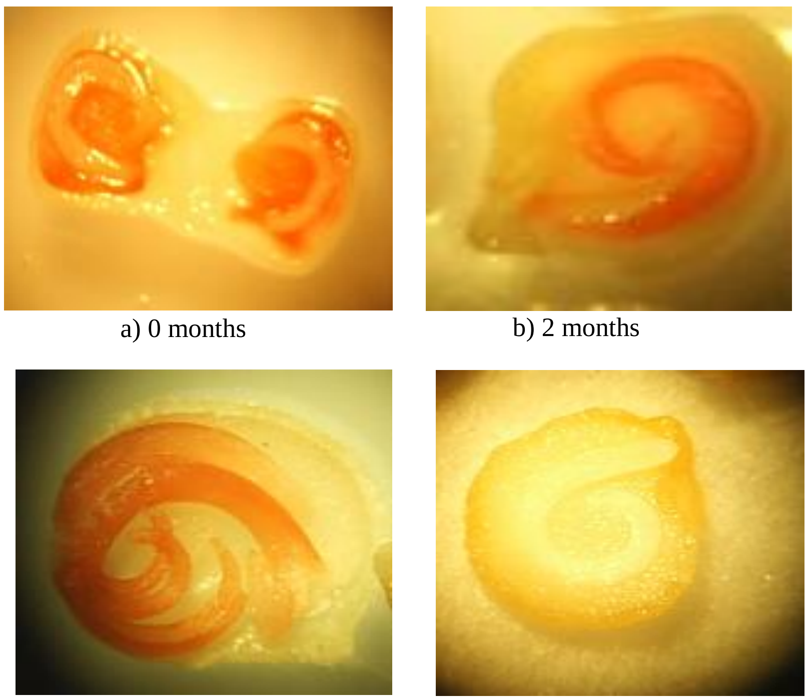
Figure 1. Absorption of water by the piquín pepper seed at different times of imbibition.

This information is important, because in practice, performing osmoconditioning prior to sowing, commonly known as seed soaking, makes this activity difficult. If the seeding is manual, the seed adheres to the hands and if it is mechanical, the seeders are designed to plant dry seeds, which is the way to plant commercial pepper seeds, such as serrano peppers, jalapeños, morrones, habaneros, etc.

Tetrazolium stain test

The tetrazolium staining test (2, 3, 5 triphenyl tetrazolium chloride) is a technique used to determine if the seeds have physiological activity. It is based on the reduction of tetrazolium by the hydrogen that is released by the activity of the hydrogenase enzymes, which are activated as a result of respiration, once the seeds have begun their germination process. Because of the reduction of tetrazolium, which is colorless, a red color is formed, which is indicative that the seed has begun its germination (ISTA, 2007, SNICS, 2017). The red coloration can appear in any part of the seed that has respiratory activity, so it is necessary to identify if this red color is present in the embryo, because in this way it can be more certain that the seed is able to germinate.

The results of this test are presented with photographs, where graphically it is shown that the seeds extracted from fresh red fruits (Figure 2a) do not present staining in the embryo, only in the endosperm of the seed, which indicates that there is activity Respiratory, not necessarily as part of the germination process, but rather as an activity of the reserve storage process. In Figures 2b and 2c corresponding to the seeds of 2 and 4 months of age, the red color is observed in the embryo, which indicates respiratory activity in this tissue, when correlating these results with the germination tests, it is concluded that respiratory activity is the result of the germination process of the seed. Finally, when looking at Figure 2d, which corresponds to the seeds of 12 months of age without benefit, no staining is observed, which indicates that there is no respiratory activity in any tissue; that is, dead seeds.



a) 0 months b) 2 months

c) 4 months d) 12 months

Figure 2. Seed staining by tetrazolium.

The seeds of 12 months and that were benefited, presented a similar staining to the seeds of 4 months of age, so that the benefit and adequate storage of the seed reduces the deterioration conserving the seed for more than a year, as it happens with seeds of commercial peppers.

Seedling production

In this test, the analysis of variance showed that the percentage of seedlings is affected by age, benefit and seed conditioning with gibberellic acid, but once the seedlings emerged no effect on their growth and development was observed and these results coincide with those obtained in the germination test.

Also, these results show that it is possible to obtain up to 96% of seedlings suitable for transplant if the seed benefits, later it is stored properly for 4 months and before planting it is conditioned with a solution of 5 000 mgL^{-1} of gibberellic acid before sowing (Table 2 and Figure 3). It was also observed that if the seed benefits and stores properly, it can maintain its viability for more than 1 year.

Table 2. Effect of age, benefit, gibberellic acid and interactions on piquín pepper seedling production.

Treatments	Mean	Error
Age 2 months	65.33 b	6.63
Age 4 months	71.33 ab	6.84
Age 12 months	45.66 b	13.51
With benefit	31.88 b	5.62
Without benefit	89.66 b	1.62
Without conditioning with gibberellic acid 5 000 mgL^{-1}	58.66 b	7.96
With conditioning with gibberellic acid 5 000 mgL^{-1}	62.88 b	8.27
Age 2 ∗ without benefit	44.33 b	2.84
Age 2 ∗ with benefit	86.33 ab	2.98
Age 4 ∗ without benefit	50.33 b	4.88
Age 4 ∗ with benefit	92.33 a	2.44
Age 12 ∗ without benefit	1 c	0.68
Age 12 ∗ with benefit	90.33 a	2.89
Age 2 ∗ without conditioning	66 ab	10
Age 2 ∗ with conditioning	64.66 b	9.65
Age 4 ∗ without conditioning	66.33 b	10.19
Age 4 * with conditioning	76.33 b	9.61
Age 12 ∗ without conditioning	43.66 bc	19.37
Age 12 ∗ with conditioning	47.66 bc	20.73
Without benefit ∗ without conditioning	29.77 b	7.46
Without benefit ∗ with conditioning	34 b	8.8
With benefit ∗ without conditioning	87.55 ab	2.25
With benefit ∗ with conditioning	91.77 a	2.24
Age 2 ∗ without benefit ∗ without conditioning	44.66 b	4.8
Age 2 ∗ without benefit ∗ with conditioning	44 b	4.16
Age 2 ∗ with benefit ∗ without conditioning	87.33 ab	4.66
Age 2 ∗ with benefit ∗ with conditioning	85.33 ab	4.66
Age 4 ∗ without benefit ∗ without conditioning	44 b	3.05
Age 4 ∗ without benefit ∗ with conditioning	56.66 b	8.35
Age 4 ∗ with benefit ∗ without conditioning	88.66 b	3.33
Age 4 ∗ with benefit ∗ with conditioning	96 a	2.3
Age 12 ∗ without benefit ∗ without conditioning	0.66 c	0.66
Age 12 ∗ without benefit ∗ with conditioning	1.33 c	1.33
Age 12 ∗ with benefit ∗ without conditioning	86.66 ab	5.2
Age 12 ∗ with benefit ∗ with conditioning	94 a	1.15

Means with different letters are statistically different (Tukey $p \leq 0.05$).

Figure 2. Piquín pepper seedling with seeds of 4 months old, benefited and conditioned with 5 000 mg L^{-1} of gibberellic acid.

The response of the piquín pepper seed to the benefit and conditioning is very similar to that of other types of commercial pepper, given that commercial pepper seeds carry a period of rest after being extracted by the dynamics of their preparation for sale. For example, broad-leaved, free-pollinated peppers seeds or varieties, in the northern region of Guanajuato, Zacatecas, Aguascalientes, are harvested during the months of september to october, and later planted in the months of november to january. Which implies that the seeds are at rest at least 2 months before planting. In the case of hybrids, harvest, profit, packaging and distribution for sale, it is estimated that it lasts more than two months.

Conclusions

The seeds extracted from fresh red piquín pepper fruits, require two months of rest to germinate and the tetrazolium stain showed that the seeds of less than two months and more than a year without conditioning do not have respiratory activity in the embryo. The benefit to the seed maintains its viability for more than a year and the conditioning prior to sowing with gibberellic acid increases germination and seedling production.

Additionally, the piquin pepper seed does not have water impermeability in the seed coat or seed coat. Finally, the percentage of germination and seedling production is obtained with seeds benefited, with 4 months of rest or age and treated or conditioned with a solution of 5 000 mgL^{-1} of gibberellic acid, 12 h before planting.

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