

**RESEARCH**
**Fatty acids and physiological quality of tomatillo (*Physalis philadelphica* Lam.) seed during natural ageing**
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In Mexico, 44 thousand hectares are planted with tomatillo or husk tomato (*Physalis philadelphica* Lam.), which occupies fourth place among the country's vegetable species. However, research on this species is scarce, especially that related to the process of seed deterioration. We studied the effects of deterioration in tomatillo seed, var. CHF1-Chapingo, stored from 2-mo up to 7 yr with no climate control,  $18.2 \pm 5$  °C and  $41 \pm 10\%$  relative humidity, on physiological and biochemical variables during germination. It was found that germination, vigor, and respiratory activity decrease significantly from the first year of storage; thus, after 7 yr, germination and vigor decreased 99%, and respiratory activity of seed after 48 h imbibition decreased 78%. Linoleic acid (unsaturated) content correlated positively with germination ( $R = 0.78^{**}$ ) and with speed of radicle emergence ( $R = 0.79^{**}$ ). Germination correlated with speed of radicle emergence ( $R = 0.99^{**}$ ) and with respiratory activity after 48 h of imbibition ( $R = 0.79^{**}$ ). Both respiratory activity and fatty acid content are involved in natural deterioration of tomatillo seed.

**Key words:** Fatty acids, natural seed deterioration, respiration.

**INTRODUCTION**

Mexico is the center of origin of species of the genus *Physalis*, which has formed part of Mexican cuisine since pre-Columbian times, especially in sauces and salads (Pichardo-González et al., 2010). The fruit of tomatillo or husk tomato (*Physalis philadelphica* Lam. or *P. ixocarpa* Brot.) is important for the preparation of regional dishes, chili sauces used to condiment prepared foods, or as an ingredient of diverse dishes (Oliver and Rojas, 2004). The tomatillo area harvested acquired importance in the 1960s and 1980s, fresh and industrialized tomatillo was exported to the USA and Canada (Pérez-Camacho et al., 2008b). In 2012 it occupied cultivated area of 44 000 ha, mean yield of  $14.4 \text{ t ha}^{-1}$  and value of USD 240 million (SIAP, 2014).

Despite its importance, research on this species is still scarce, especially that related to seed quality and its

deterioration (Pichardo-González et al., 2010). According to Pérez-Camacho et al. (2008a), germination of tomatillo seed stored without refrigeration decreases 9% annually. This aspect is important to consider in establishing commercial seed storage facilities, genetic improvement programs or germplasm banks. The study of physiological and biochemical processes that occur during tomatillo seed germination could explain factors involved in its deterioration.

Seed of any species is at its highest level of vigor and germination potential at physiological maturity. At this point, a continuous and irreversible process of deterioration begins and progresses until the seed loses its capacity to germinate (Carrillo-Salazar et al., 2011). Seed deterioration can be understood as a series of changes over time that affects its vital functions and performance, up to its death. During storage, mechanisms of seed deterioration decrease germination, speed of seedling growth and tolerance to adverse conditions (Bewley et al., 2013).

The rate of deterioration varies among species and varieties. It also depends on environmental conditions and storage time (Walters et al., 2010). Under conditions of high temperatures and high relative humidity, this rate is high. In a batch of seed, deterioration causes discoloration, low germination and delayed seedling growth, abnormal seedlings and low yield (Bewley et al., 2013). In individual seed, respiration and enzymatic activity decrease and membranes are damaged (Kaewnaee et al., 2011). Moreover, seed deterioration involves other

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biochemical and biophysical changes, such as lipid peroxidation and stress caused by oxidative free radicals (Walters et al., 2010). These factors contribute greatly to seed deterioration because they affect membrane structure and function by inactivating them and altering their permeability. Seed deterioration is also associated with a reduction in reserve carbohydrates, which could result in insufficient respiratory substrates for germination or in the inability to use them; thus, a symptom of seed deterioration is a significant decrease in the rate of respiration (Pérez-Camacho et al., 2008a; Pichardo-González et al., 2010).

This study assessed deterioration of tomatillo seed stored without climate control using physiological and biochemical variables during germination.

## MATERIALS AND METHODS

The tomatillo seed used was the var. CHF1-Chapingo, produced in the field during the spring-summer production cycles 2005, 2008, 2009, 2011, and 2012 (stored for 7, 4, 3, and 1 yr, and for 2-mo) in Chapingo (98°53' N, 19°29' W; 2250 m a.s.l.), Mexico. Crop production management was the same every year. Transplanting in the field was during the first week of April at a density of 30 000 plants ha<sup>-1</sup>, and fruits were harvested the second week of June. Seed extracted from the fruit was dried at room temperature in the shade and processed: cleaned and selected by size in an air-screening machine (LALS-type, Kamas Industri AB, Vellinge, Sweden). Processed seed was stored in paper bags in a storeroom with no climate control (18.2 ± 5 °C and 41 ± 10% RH).

To determine initial moisture content, a seed sample from each period of storage was weighed, dehydrated for 72 h at 72 °C in an oven (Central Scientific; Cenco Instruments Corporation, Chicago, Illinois, USA) and weighed again. Moisture content was determined as the difference between fresh and dry biomass weight.

### Content of fatty acids

For each storage period, seeds were analyzed to determine fatty acid content. Fatty acids were extracted following the method of Priestley and Leopold (1979), and transesterification was done with the Morrison and Smith method modified by Priestley et al. (1985). Assessment of the treatments (seed ages) was performed under a completely random design with three replicates of 20 mg seed.

Composition of fatty acids was analyzed in a gas chromatograph coupled with a mass spectrometer (model MAT GCQ GC970894 MS100412, Thermo Finnigan, Bremen, Germany). Chromatographic conditions were the following: particle dispersion injection, injector temperature 200 °C in an oven with a scheduled temperature gradient from 100 °C (3 min from initiation) to 250 °C (for 4 min) with 5 °C increases per minute. The gas chromatograph column was type DB-1 (dimethylpolysiloxane). Helium was used as the gas

carrier at a speed of 35 cm s<sup>-1</sup>. Conditions of the mass detector were the following: transfer line temperature 250 °C, interval 20 to 400 mass-charge (m/z) reactions, positive polarity, ion source temperature 200 °C and electron ionization impact at 70 eV. A 2 μL volume of the organic phase extract was injected. The fatty acids were identified by the retention times of methyl standards and with the fragmentation patterns obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, Maryland, USA) reference libraries.

### Germination and vigor

A germination test was conducted with the batches of seed of the years assessed on filter paper with three replicates of 50 seeds, following a completely random design. The seeds in Petri dishes with moistened filter paper were placed in a germinator SD8900 (Seedburo Equipment Co., Des Plaines, Illinois, USA) at 25 ± 1 °C, with 12 h light and 12 h darkness for 24 d (ISTA, 2004). With this test, percentage of germination (G) and speed of radicle emergence (SRE) were assessed; the latter was the expression of seed vigor and was calculated with the Maguire (1962) formula, which is based on the number of radicles emerged every 24 h.

### Measurement of respiratory activity

In seeds stored for different periods of time, respiratory kinetics was measured with a gas chromatograph (HP 5890, Hewlett Packard, Palo Alto, California, USA) 48 h after initiating the process of imbibition-germination. Measurements were replicated three times on 50 seeds, under a completely random design. The 50 seeds were placed in 36 mL glass jars, which were hermetically sealed with a plastic cover. After 1 h in the sealed jars, 1 mL of air with CO<sub>2</sub> was extracted with an insulin syringe and injected into the gas chromatograph. The CO<sub>2</sub> released by respiration was calculated as the area under the curve of each peak, and the data were transformed into mL h<sup>-1</sup>.

### Statistical analysis

Statistical analyses were performed with SAS software (2002, SAS Institute, Cary, North Carolina, USA). Before analysis, data of the variables measured in percentage were transformed with the arc-sine  $\sqrt{X}/100$ . Comparison of means tests (Tukey;  $p \leq 0.05$ ) were done as well as a correlation analysis with germination percentage, speed of radicle emergence, and respiration kinetics. The graphs and curve equations were processed on a Microsoft Excel (Microsoft Corporation, Redmond, Washington, USA) spreadsheet.

## RESULTS AND DISCUSSION

Initial moisture content of the assessed seeds of different ages was 6% on average. This value was not low and did not interfere with the measured variables because in dry

seeds biological processes are much slower than in moist seeds.

### Fatty acids content

Transesterification performed with gas chromatography coupled with a mass spectrometer revealed the presence of the following fatty acids in tomatillo seeds stored for different periods, based on retention times: Palmitic, linoleic, oleic, and stearic acids. The presence of other acids was not detected in seeds since the method used is specific for fatty acids (Priestley and Leopold, 1979; Priestley et al., 1985).

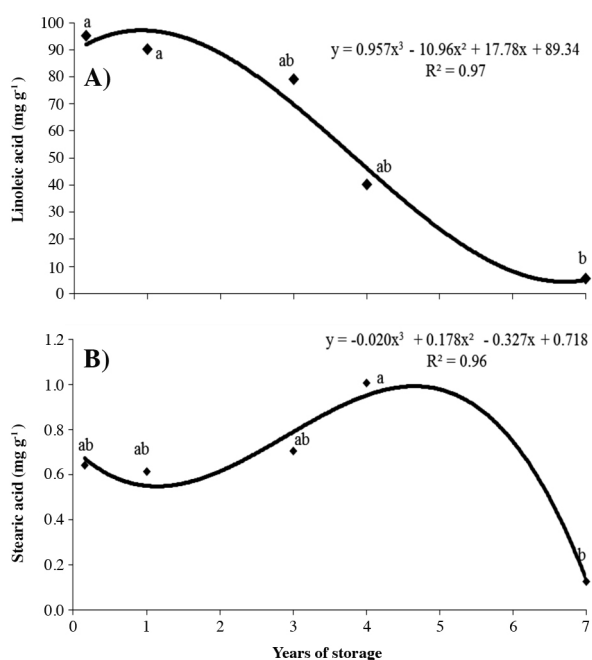
It was found that seed age affected ( $p \leq 0.05$ ) contents of linoleic and stearic acids ( $p \leq 0.01$ ), as well as germination (G), speed of radicle emergence (SRE), and respiratory activity (Table 1). Unlike G, SRE and respiratory activity, which had low coefficients of variation ( $\leq 16\%$ ), fatty acids had high coefficients of variation ( $> 27\%$ ) attributed to the heterogeneity among seed batches in terms of fatty acid composition.

Of the fatty acids quantified, linoleic acid was in the highest concentration (83.7% to 95.2%), followed by oleic acid (2.8% to 6.7%), palmitic acid (1.4% to 7.7%), and stearic acid (0.6% to 2.2%). The first two are unsaturated and the other two are saturated; that is, in tomatillo seed there is a larger proportion of unsaturated than saturated fatty acids. Similar results were found by Pichardo-González et al. (2010) in seed of same tomatillo variety used in our study. Likewise, Walters et al. (2005) found similar results when they quantified fatty acids in sunflower seeds (*Helianthus annuus* L.). The fatty acids of cotyledons are predominantly linoleic and oleic acids. Also, Gómez-Tejero et al. (2006) found a larger proportion of unsaturated fatty acids in seeds of *Swietenia macrophylla* King and other forest species.

The contents of palmitic (saturated) and oleic (unsaturated) acids were not significantly different in all seeds stored for different numbers of years, with average values of 1.65 and 62.03 mg g<sup>-1</sup> seed, respectively. In contrast, contents of linoleic (unsaturated) and stearic (saturated) acids were significantly different over the years of storage. Linoleic acid (Figure 1A) was present in higher concentrations during the first 4 yr of storage, but in the seventh year there was a significant reduction of 94% (5.4 mg g<sup>-1</sup> seed) relative to the seed stored for 2-mo. Although 5 and 6-yr-old seeds were not assessed, a

rapid reduction trend is noted as of 3 yr of storage (Figure 1A). This decrease can be attributed to the formation of free radicals that cause lipid peroxidation since linoleic acid easily converts into an oxidized or hydroperoxic fatty acid (Melo and Cuamatzi, 2008). In this respect, Sacandé et al. (2000) deem that the presence of two double links in C make it lighter and polar and, consequently, more susceptible to attack by free radicals that decrease its content in the seed.

In this study, it was not possible to confirm the formation of free radicals in the stored seeds since direct evidence from paramagnetic electron resonance was lacking (Hepburn et al., 1986). Also, there was no confirmation of fatty acid by-products, such as malondialdehyde (Wilson and McDonald, 1986). Nevertheless, the decrease in the unsaturated linoleic acid content may be evidence of lipid peroxidation by free radicals.



Values with the same letter are not significantly different according to Tukey's test ( $p \leq 0.05$ ).

**Figure 1.** Fatty acid contents in tomatillo seeds naturally deteriorated for up to 7 yr in storage with no climate control. A) Linoleic acid and B) stearic acid.

**Table 1.** Mean squares and statistical significance of fatty acid contents, germination, speed of radicle emergence, and respiratory activity of tomatillo seed stored for up to 7 yr without climate control.

SV	DF	PAL	LIN	OLE	ST	G	SRE	RESP
		mg g <sup>-1</sup> seed				%	rad d <sup>-1</sup>	mL CO <sub>2</sub> g <sup>-1</sup> h <sup>-1</sup>
Age	4	1.9 ns	4399.5*	10.6 ns	0.3*	1945.7**	142.3**	0.07**
Error	8	1.0	743.1	3.8	0.04	22.4	1.5	0.001
CV, %		61.8	43.9	63.5	34.7	10.5	11.6	15.8

SV: sources of variation; DF: degrees of freedom; PAL: palmitic acid; LIN: linoleic acid; OLE: oleic acid; ST: stearic acid; G: germination; SRE: speed of radicle emergence; RESP: respiration after 48 h imbibition; CV: coefficient of variation.

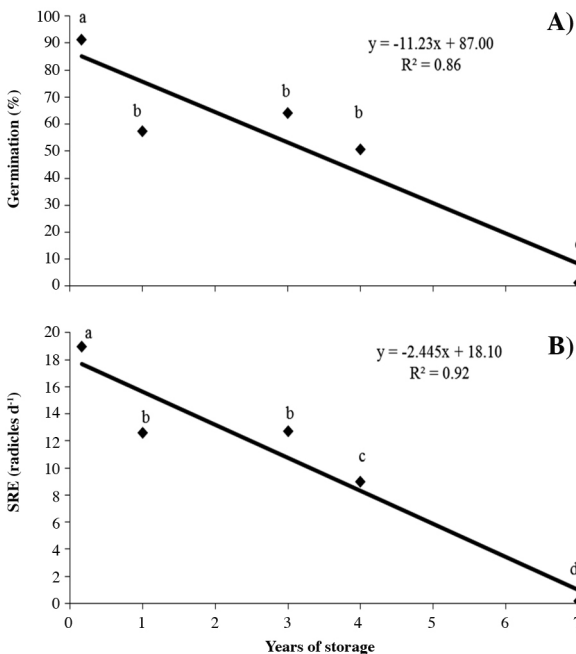
\*, \*\*Significant at  $p \leq 0.05$  and  $p \leq 0.01$ , respectively; ns: non significant.

In a similar way, stearic acid (Figure 1B) was present at an average concentration of 0.7 mg g<sup>-1</sup> seed from 2-mo up to the fourth year of storage, but in the seventh year the concentration decreased significantly to 0.13 mg g<sup>-1</sup> seed, a reduction equivalent to 87% relative to the seed stored for 2-mo. This significant reduction in stearic acid (saturated) content could be due to the activity of enzymes of the desaturase type (Sacandé et al., 2000).

The results obtained in our experiment contrast with findings of Pichardo-González et al. (2010), who did not detect any significant decrease in the fatty acid contents of tomatillo seeds artificially deteriorated with the technique of accelerated aging by combining different temperatures (45 and 50 °C) and relative humidity (55%, 65%, and 75%) for 30 d storage.

### Germination and vigor

Tomatillo seed stored for 2-mo had the highest rate of germination (91%, Figure 2), a percentage that is within the norm for commercial seeds (SNICS, 1975). One year later, germination decreased 44% ( $p \leq 0.05$ ), relative to the 2-mo-old seed, and from the first to the fourth year there were no differences among ages, with an average reduction of 37%. This is below the norm (SNICS, 1975). Storage for 7 yr was lethal; germination decreased to 1%. These results show that germination decreases with time of storage. This is similar to behavior reported by Pérez-Camacho et al. (2008a) in maize (*Zea mays* L.) and Carrillo-Salazar et al. (2011) for tomatillo seeds.



Values with the same letter are not significantly different according to Tukey's test ( $p \leq 0.05$ ).

**Figure 2.** Effect of storage time on physiological characteristics of tomatillo seed. A) Germination and B) speed of radicle emergence (SRE).

In tomatillo seed, Pérez-Camacho et al. (2008a) found an 8.7% yearly germination loss of seed stored with no climate control. In our study, a higher loss was found, 11.2% per year. Although storage conditions used in the above study and ours were the same, differences may be due to the number of years of assessment: 5 vs. 7 yr of our study.

The results obtained on natural aging of tomatillo seeds show that linoleic fatty acid content decreases over storage time, similar to germination. However, the model of the germination curve is linear (Figure 2A), while that of fatty acid content is polynomial (Figure 1A). Linoleic acid continually decreases in all years, but during the first years the decrease is slow; the fourth year decline is rapid. It can thus be inferred that small decreases in the content of this fatty acid can significantly decrease germination. Lipid peroxidation associated with aging has been studied in soybean (*Glycine max* (L.) Merr.), tomato (*Lycopersicon esculentum* Mill.), and peanuts (*Arachis hypogaea* L.) (Wilson and McDonald, 1986). These studies documented decreases in unsaturated fatty acids and germination. In the case of artificially deteriorated tomatillo seed, Pichardo-González et al. (2010) observed a reduction in germination and vigor, with no significant decrease in fatty acid contents. This could indicate that the decrease in germination in naturally deteriorated seeds, such as those in our study, does not have the same cause as that in artificially deteriorated seeds. In this respect, Halmer (2000) indicated that the accelerated aging technique causes an abnormal state in terms of nucleic acid metabolism and membrane structure. Thus, it is not recommended as a method in assessing seed vigor, mainly with crop species with large seeds (grains); in species with small seeds, such as flower, vegetables and grasses, correlations between artificial and natural aging are low.

Seed vigor, assessed with the SRE index, showed the highest value for the seed stored for 2-mo, with 18.9 radicles d<sup>-1</sup> (Figure 2B). After 1 yr storage there was a 34% reduction, relative to seeds stored 2-mo, equivalent to 12.5 radicles d<sup>-1</sup>. The decrease continued until in the seventh year; only 0.17 radicles d<sup>-1</sup> were observed, equivalent to a 99% reduction and an annual loss of 2.4%. This value is lower than that reported by Pérez-Camacho et al. (2008a), who found an annual reduction of 11.1% in tomatillo seed vigor.

Speed of radicle emergence had the same behavior as germination. Constant decreases were observed in both variables from the first up to the seventh year of storage, showing that the two are highly correlated. According to Bewley et al. (2013), the first quality component to show signs of deterioration is seed vigor, followed by a decrease in germination and, finally, death of the seed.

### Respiration

The seed stored for 2-mo had the highest respiratory activity after 48 h imbibition with 0.5 mL CO<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>

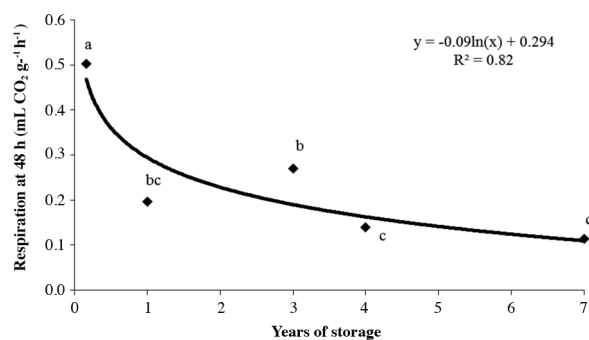
(Figure 3). After 1 yr storage, respiration decreased ( $p \leq 0.05$ ) during germination to  $0.19 \text{ mL CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ , or 38%, relative to 2-mo-old seed. In older seeds, stored for a longer time, rate of respiration was equal to or lower ( $p \leq 0.05$ ) than that of 1-yr-old seed. The tomatillo seed respiratory activity curve shows that the rate of respiration decreases rapidly the first year; this can be attributed to seed deterioration. In tomatillo seeds of the same variety (CHF1-Chapingo), Pérez-Camacho et al. (2008a) reported that respiration decreases significantly until after the second year of age.

Walters et al. (2010) mention that seed deterioration is associated with changes in metabolism, such as a decrease in carbohydrates that occurs with seed age. This could result in insufficient respiratory substrates for germination or in the inability to use them. For this reason, a reliable indicator of seed deterioration is the reduction in its rate of respiration. Bewley et al. (2013) attribute the decrease in respiratory activity in old seeds to a decrease in translocation of the necessary substrates to the embryo axis and to the loss of stored reserves. In tomatillo seed Pérez-Camacho et al. (2008a) and Pichardo-González et al. (2010) found that a reduction in reserves largely explains losses of germination and vigor in deteriorated seed.

This drastic reduction in respiration of 1-yr-old seeds probably became manifest due to natural storage conditions without climate control ( $18.2 \pm 5 \text{ }^\circ\text{C}$  and  $41 \pm 10\% \text{ RH}$ ), which are not suitable for storage of these seeds.

### Physiological aspects of deterioration

The result of this study showed that seed, only 2-mo-old, had higher values for germination, speed of radicle emergence, respiration, and linoleic acid content. In contrast, germination, vigor and linoleic acid content of 7-yr-old seed had very low values (nearly 0). For this reason, these characteristics can be considered symptoms of tomatillo seed deterioration. In a similar



Values with the same letter are not significantly different according to Tukey's test ( $p \leq 0.05$ ).

**Figure 3.** Respiration at 48 h of imbibition in tomatillo seed stored up to 7 yr without climate control.

manner, Pérez-Camacho et al. (2008b) in tomatillo seed of the same variety (CHF1-Chapingo) and Bewley et al. (2013) in seeds of several species point out that seeds are at their highest level of vigor and germinating potential at physiological maturity. At this point a continuous irreversible process of deterioration begins and continues until the seed loses its ability to germinate. Moreover, Walters et al. (2010) mention that seed quality diminishes over time and the rate of deterioration varies with species and depends on environmental conditions during storage.

The correlation analysis showed that germination correlated positively with speed of radicle emergence (vigor) ( $R = 0.99^{**}$ ), respiratory activity after 48 h of imbibition ( $R = 0.79^{**}$ ), and linoleic fatty acid content ( $R = 0.78^{**}$ ) (Table 2). Speed of radicle emergence correlated positively with respiration ( $R = 0.81^{**}$ ) and linoleic acid content ( $R = 0.79^{**}$ ).

This confirms that respiratory activity in tomatillo seed is closely related to germination and vigor, indicators of seed physiological quality. Thus, a decrease in physiological quality can be explained by the decrease in respiration, which translates into less energy available for germination processes (Bewley et al., 2013). It is also inferred that the kinetics of respiratory activity after 48 h could be an early test of physiological quality of a batch of seeds since it is linearly associated with germination and speed of radicle emergence. Sundstrom and Edwards (1989) proposed respiratory activity of chili seeds (*Capsicum annum* L.) as a reliable indicator of seed vigor since high rates of respiration correlated with high germination rates and rapid radicle emergence. Moreover, Tatić et al. (2012) observed that deterioration of soybean seed reduced respiration rate and speed of radicle emergence, behavior associated with seed vigor.

As described above, linoleic acid content decreases over storage time. However, the behavior of this acid, from the first to the fourth year, is not like that of germination and vigor, whose values decrease linearly as storage time lengthens until values are practically zero after 7 yr. In new seed and seed stored for up to 3 yr, small decreases in linoleic acid content are reflected in low germination and vigor. It is likely that this decrease was caused by seed deterioration due to free radicals from oxidized linoleic acid, which may have affected membrane and

**Table 2.** Coefficients of correlation between variables assessed in tomatillo seed stored for different numbers of years with no climate control.

Variable	SRE	PAL	LIN	OLE	ST	RESP
GER	0.99**	0.35	0.78**	0.44	0.50	0.79**
SRE		0.34	0.79**	0.43	0.44	0.81**
PAL			0.25	0.69**	0.48	0.05
LIN				0.69**	0.39	0.54*
OLE					0.45	0.09
ST						0.08

GER: Germination; SRE: speed of radicle emergence; PAL: palmitic acid; LIN: linoleic acid; OLE: oleic acid; ST: stearic acid; RESP: respiration after 48 h of imbibition.

\*, \*\*Significant at  $p \leq 0.05$  and  $p \leq 0.01$ , respectively.

reserve lipids, resulting in damage to the membranes and to reserve substances, as Kaewnaee et al. (2011) have stated.

It is inferred that both the decrease in seed respiration caused by loss of reserves and peroxidation of linoleic acid (unsaturated) in seed cells contribute to tomatillo seed deterioration under natural storage conditions. This was reflected in low germination rate and vigor as storage time lengthened.

## CONCLUSIONS

Tomatillo seed stored at  $18.2 \pm 5$  °C and  $41 \pm 10\%$  RH for up to 7 yr lost its germinating capacity at an annual rate of 11.2%, a loss of 99% by the seventh year. Speed of radicle emergence decreased to a rate of 2.4 seedlings d<sup>-1</sup>. Linoleic acid decreased constantly as storage time lengthened, but with kinetics different from that of germination; during the first years of storage there was no significant variation in the content of this acid, but in the seventh year there was a decrease of 94% relative to 2-mo-old seeds. In the first year of storage, respiration decreased significantly to 0.19 mL CO<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup> (62 %, relative to 2-mo-old seed). Germination correlated positively with speed of radicle emergence, respiratory activity after 48 h of imbibition, and linoleic acid content. For this reason, it is inferred that both respiratory activity and fatty acid content are involved in natural deterioration of tomatillo seed, reflected in reduced germination and vigor over storage time.

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