Evaluation of seed morphology, seedling genetic variation, and components for seed storage of Agave landraces of commercial interest. V.1

DOI
dx.doi.org/10.17504/protocols.io.6qpvr6j12vmk/v1

VERSION 1
DEC 13, 2022

Jesus A Jimenez-Torres[1],
Zurisadai Monroy-Gonzalez[1],
Juana Juarez-Muñoz[1]

[1]Universidad Autónoma del Estado de Hidalgo

Jesus A Jimenez-Torres

ABSTRACT

Sexual propagation of Agave plants is an incipient cultivation method, these plants withstand drought and adverse growing conditions; therefore, research on Agave's diversity, seed processing and storage, could support its cultivation on marginal lands. The aim of this work was to evaluate seed morphology, germination, and seedling genetic diversity of six seed origins (species*provenances) of Agaveplantscollected in five provenances from Mexico. Seed longevity was evaluated in two seed origins after a ten-year storage period. Seed morphology and seedling genetic variation results demonstrated intra- and interspecific variation within *Agave salmiana* and the other seed origins, respectively. After a ten-year storage period seed germination of two *A. salmiana* seed origins remained relatively stable, storage conditions and seed variables of this work can serve as reference parameters for future analyses. To the best authors' knowledge this is the first report of Agave's seed longevity evaluation after a ten-year storage period.

DOI

dx.doi.org/10.17504/protocols.io.6qpvr6j12vmk/v1

DOCUMENT CITATION

Jesus A Jimenez-Torres, Zurisadai Monroy-Gonzalez, Juana Juarez-Muñoz 2022. Evaluation of seed morphology, seedling genetic variation, and components for seed storage of Agave landraces of commercial interest.. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.6qpvr6j12vmk/v1
Version created by Jesus A Jimenez-Torres

KEYWORDS

Agave americana, Agave mapisaga, Agave salmiana, seed longevity, ten-year storage

CREATED

Sep 06, 2022

LAST MODIFIED

Dec 13, 2022

DOCUMENT INTEGER ID

69604

Evaluation of seed morphology, seedling genetic variation, and components for seed storage of Agave landraces of commercial interest.

Jesus A. Jimenez-Torres *; Zurisadai Monroy-Gonzalez; Juana Juarez-Muñoz
*Corresponding author: tlacametl@gmail.com

1.1 Biological Material

1.1 Seed collection sites

Green-yellowish capsules of *Agave mapisaga* and *A. salmiana*, and fully mature brown capsules of *Agave americana*, were collected during summer 2012 (June - July) from flowering stalks of individual plants located in five provenances of Hidalgo State, Mexico (Table 1). The green-yellowish capsules were air-dried at room temperature until reached brown colour. Thereafter, all capsules were mechanically opened, and seeds removed, a batch of one hundred seeds of each seed origin were stored at 8 ºC for the evaluation of seed relative weight, morphology, and germination.
The interaction of species*provenance was considered as a seed origin. The climatic data and geographical features of Metepec, Tlajomulco and Tlaxiaca provenances can be found in the previous work of Jimenez-Torres et al. (2021). The germplasm of all the seed origins evaluated were collected from maguey pulquero plantations under the “milpa” agroecosystem (Zizumbo-Villarreal et al. 2012), with seasonal farming (late spring- autumn). Sauz Xathe provenance in Atotonilco El Grande municipality, is located in the southern of the Sierra Madre Oriental Mountain range, which is a microhabitat within the Meztitlan Canyon biosphere reserve, with sedimentary soils and temperate semi-arid climate (INEGI, 1997).

Table 1. Location, species, geographic and climatic data of seeds collected at Hidalgo state, Mexico.

A	B	C	D
Seed origin	Provenance/ Species	Geolocation; altitude (m.a.s.l.)	*Mean temperature (min-max); pr
SalmMet	Metepec / *A. salmiana*	N 20º 15’ 31” W 98 º 21’ 03”; 2	13.4 -23.4 ºC; 623 mm / 1,539 mm
SalmMetβ	Metepecβ / *A. salmiana*	N 20 º 14’ 17” W 98 º 21’ 16”; 2	13.4 -23.4 ºC; 623 mm / 1,539 mm
SalmTlajo	Tlajomulco / *A. salmiana*	N 19 º 54’ 14” W 98 º 33’ 04”; 2	4.4-24.1 ºC; 529 mm / NA
SalmTlax	Tlaxiaca / *A. salmiana*	N 20 º 07’ 33” W 98 º 50’ 02”; 2	7.6-22.1 ºC; 444 mm / 1,855 mm
MapsMet	Metepec / *A. mapisaga*	N 20 º 15’ 31” W 98 º 21’ 03”; 2	13.4 -23.4 ºC; 623 mm / 1539 mm

Citation: Jesus A Jimenez-Torres, Zurisadai Monroy-Gonzalez, Juana Juarez-MuÃÂ±oz Evaluation of seed morphology, seedling genetic variation, and components for seed storage of Agave landraces of commercial interest.
https://dx.doi.org/10.17504/protocols.io.6qpvr6j12vmk/v1

A	B	C	D
AmerSauz	Sauz Xathe / *A. americana*	N 20 º 20’ 20” W 98 º 45’ 59”; 1	10.3-22.7 ºC; 789.4 mm / 1,412 mı

*Average of annual data over a three-decade period from 1981 to 2010 (Conagua, 2021). NA = not available.

2. Methods

2.1 Seed morphometric and germination evaluation

One hundred seeds of each seed origin were selected randomly comprising a batch; each seed was weighted with analytical balance and labelled individually for morphometric analyses. Seeds of each batch were placed according to the individual label order in a blank paper using a one-centimetre line as reference. Thereafter, a digital image of the whole batch was obtained with a steady camera Sony DSRL-A100; images were evaluated with software Image-Pro plus ver. 6.0 (2006), the reference line and seeds were determined into the software parameters; thereafter, area, roundness, width, and length were measured automatically by the software settings.

Germination was evaluated in sterile conditions. All seeds of each batch were placed over a paper towel into petri dishes, and then 8 mL of sterile water were added. Petri dishes were sealed with Parafilm and introduced into an incubation chamber at temperature 28 C for 24 hrs, with light/dark period of 16/8 hrs. Germination was evaluated daily; a germinated embryo was considered when 2 mm approx. of radicle was observed.

2.2 Genetic analyses

2.2.1 Plant material and DNA extraction

Seeds of one individual of each seed origin were disinfested with a sodium hypochlorite solution 30 % (v/v) for 10 min in an orbital shaker; then, the seeds were rinsed with sterile deionized water. Thereafter, seeds were germinated in similar conditions as the germination experiment section 2.1, when the seedlings reached 5 cm length the seed coat and root were discarded, and the cotyledons were used for DNA extraction. Then, the cotyledons were lyophilized for 24 h at -40 ˚C and 2000 millibars. DNA from six cotyledons of each seed provenance was isolatedby the method of Dellaporta et al. (1983).

For DNA purity determination an aliquot (5 µL) of concentrated DNA was mixed with 595 µL of Tris-HCl 10 mM at pH 8 and EDTA 1 mM, then the sample was analyzed with a spectrophotometer (Spectronic2000)at 260 and 280 nm. The quotient between the absorbance obtained (abs. at 260 nm / abs. at 280 nm) estimated the nucleic acid purity, which values within 1.8 and 2.0 indicated a high DNA purity (Wilfinger et al. 1997).

DNA integrity was assessed by electrophoresis with 0.8% (w/v) agarose gel stained with ethidium bromide.

2.2.2 Randomly amplified polymorphic DNA (RAPDs)

The RAPDs analysis was performed with eight different random 10-mer primers (Table 2) (Carl Roth GmbH + Co. KG). The polymerase chain reaction (PCR) was performed with areaction volume of 25 µL containing 200 µM of DNTPs, 10X Taq polymerase buffer, 20 ρmol of primers (Table 2), 3 mM of $MgCl_2$,1.5 units Taq DNA polymerase (Gibco BRL) and 100 ng of DNA. The PCR reaction was performed using a thermocycler Techne TC 412. The PCR program consisted of an initial denaturation cycle at 94 °C for 1 min, followed by 38 cycles: denaturalization at 95 °C (for 30 s from cycle 1 to 3 and 15 s from cycle 4 to 38), annealing at 35 °C for 30 s and extension at 72 °C for 1.30 min.The DNA amplified products were separated in 1.2% (w/v) agarose gel-stained with ethidium bromide and visualized in a Gel Doc 1000 (Bio-Rad). The molecular weight of the amplified DNA fragments was determined comparing to a 100 bp ladder (Gibco BRL).

Table 2. Sequences of primers used for genetic variation analysis of Agave seedlings

A	B	C

Citation: Jesus A Jimenez-Torres, Zurisadai Monroy-Gonzalez, Juana Juarez-MuÃÂ±oz Evaluation of seed morphology, seedling genetic variation, and components for seed storage of Agave landraces of commercial interest.
https://dx.doi.org/10.17504/protocols.io.6qpvr6j12vmk/v1

A	B	C
No.	Primer	sequence (5'-3')
1	ROTH-H 01	GGT CGGAGA A
2	ROTH-H 13	GAC GCC ACA C
3	ROTH-H 16	TCT CAG CTG G
4	ROTH-G 03	GAG CCC TCC A
5	ROTH-G 04	AGC GTG TCT G
6	ROTH-G 05	CTG AGA CGG A
7	ROTH-G 06	GTG CCT AAC C
8	ROTH-G 08	TCA CGT CCA C

2.2.3 DNA data analysis

A binary matrix of bands indicating presence (1) or absence (0) was built with amplified DNA fragments of the Agave seedlings. Multivariate analysis was performed constructing a dendrogram with the binary matrix of bands using the Ward's method (Trejo et al. 2020) with the statistical software Statgraphics 19.

2.3 Seed longevity evaluation after a ten-year storage period

2.3.1 Seed storage conditions

Same germplasm of *A. salmiana* collected during summer 2012 (June - July) from second location of Metepec (SalmMetβ) and Tlaxiaca (SalmTlax) (Table 1) were preserved at room temperature under cool-dry dark conditions from August 2012 to April 2022 (117 months), which was rounded up to a ten-year period, in a facility from Tlajomulco, Zempoala Municipality, Hidalgo State, Mexico.

Seed processing before storage: the seeds were maintained at room temperature and ambient relative humidity while processing, vain seeds and impurities were removed, and the seeds were placed into airtight sealed glass jars (1 L volume) then placed into plastic coolers.

The storage ambient temperature was of the Tlajomulco provenance (Table 1), normal climate data (1951-2010) of Zempoala climatic station located at 12 km east from Tlajomulco, both in the same ecoregion, reports mean temperature of 14 °C, mean warmer temperatures ranging from 24 to 26 °C during March-April, and colder temperatures ranging from 4 to 2 °C during November-January (CONAGUA, 2021). During the warm season the storage room reduced thermal sensation from ambient conditions as it was a well-ventilated construction built with stone walls (thickness 50 cm, height 3 m) with clay roof tiles.

For comparative purposes, brown capsules from same *A. salmiana* plantation of Tlajomulco provenance were collected in September of 2017 (SalmTlajo17) these were processed similarly as described previously and the seeds were stored in same conditions as the seeds from 2012.

2.3.2 Seed longevity evaluation

Seed longevity was evaluated by maximum seed germination in SalmMetβ, SalmTlax (both stored since 2012) and SalmTlajo17 (stored since 2017) using the same ambient conditions mentioned in section 2.1 (temperature 28 °C for 24 hrs., light/dark period 16/8 hrs.), the experimental unit was 25 seeds per seed origin placed into petri dishes (n = 4).

Citation: Jesus A Jimenez-Torres, Zurisadai Monroy-Gonzalez, Juana Juarez-MuÃÂ±oz Evaluation of seed morphology, seedling genetic variation, and components for seed storage of Agave landraces of commercial interest.
https://dx.doi.org/10.17504/protocols.io.6qpvr6j12vmk/v1

Seed relative weight was measured in four batches after opening the glass jars, per batch the seeds were counted until reaching a relative weight close to one gram (exact weight of analytical balance was used for calculations). Thereafter, seed dry weight was measured as follows: seed batches were dried at 100 °C ± 3 until constant weight, and seed moisture content determination was calculated according to Ellis (1990).

2.4 Statistical analysis

The results of seed morphology and seed longevity experiments were assessed by ANOVA and post-hoc means comparison by Tukey's test ($p \leq 0.05$). Multivariate analysis of seed morphology results was performed by Andrews curves. The analyses were performed with the statistical software Statgraphics 19.

References

CONAGUA, 2021. Normal weather information of Hidalgo state climatic stations. Information retrieved online from: https://smn.conagua.gob.mx/es/informacion-climatologica-por-estado?estado=hgo.

Dellaporta SL, Wood J, Hicks JB. 1983. A plant DNA minipreparation. Version II. Plant Molecular Biology Reproduction 1: 19-21.

Ellis RH, Hong TD, Roberts EH, Tao K-L. 1990. Low Moisture Content Limits to Relations Between Seed Longevity and Moisture. Ann Bot. 65(5):493–504. https://doi.org/10.1093/oxfordjournals.aob.a087961

INEGI- National Institute of Geography, Statistics and Informatics (1997). Geographical information of Atotonilco el Grande municipality, Hidalgo state, Mexico. Retrieved online:http://internet.contenidos.inegi.org.mx/contenidos/productos/prod_serv/contenidos/espanol/bvinegi/productos/historicos/1334/702825928018/702825928018_1.pdf

Jimenez-Torres JA, Peña-Valdivia CB, Padilla-Chacon D, Garcia-Nava R. 2021. Physiological and biochemical responses of *Agave* to temperature and climate of their native environment. Flora 278: 151797. https://doi.org/10.1016/j.flora.2021.151797

Trejo L., Reyes M., Cortes-Toto D., Romano-Grande E., Muñoz-Camacho L.L. 2020. Morphological diversity and genetic relationships in pulque production Agaves in Tlaxcala, Mexico of unsupervised learning and gene sequencing analysis. Front. Plant Sci., 11:524812. https://doi: 10.3389/fpls.2020.524812.

Wilfinger W.W., Mackey K., and Chomczynski P. 1997. Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. Biotechniques 3(22):474-481.

Zizumbo-Villarreal D., Flores-Silva A., and Colunga-Garcia M.P. 2012. The archaic diet in Mesoamerica: Incentive for milpa development and species domestication. Economy Botany, 66(4): 328-343.

Citation: Jesus A Jimenez-Torres, Zurisadai Monroy-Gonzalez, Juana Juarez-MuÃÂ±oz Evaluation of seed morphology, seedling genetic variation, and components for seed storage of Agave landraces of commercial interest. https://dx.doi.org/10.17504/protocols.io.6qpvr6j12vmk/v1