

(Online First) An approach to the model for conservation of Central Mexico native grapevines

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ABSTRACT

Several and diverse native populations of wild grapevines have been reported growing in the States of Puebla, Mexico and Morelos, Central Mexico. After collection, successfully rooted vines were planted in Zumpahuacán, State of Mexico, and since 2013, their fruit were analyzed, mainly in relation to berry-weight and sweetness. Recently, the seed oil content was analyzed. Most berries harvested in 2013, 2014 and 2015 weighted less than 1 g and only berries of accession Puebla-41 (P-41) weighted nearly 2.5 g. On the other hand, most berries had lower than 18°B. Four accessions, three native of Puebla and one from Morelos had the higher fruit production (2.5, 1.5 and 1.4; and 0.68 kg per plant) and might be the genetic material proposed to be planted in Zumpahuacán, Mexico. Seed oil analysis confirmed the presence of linoleic acid as the main fatty acid in Central Mexico wild grape seed oil. Nowadays, the extraction of seed oil in Central Mexico native grapes seems to be the better alternative to interest locals and agro-industrial related companies in conserving and cropping this plant genetic resource.

Keywords: Berry size; fruit genetic resource; germplasm bank; oil quality; *Vitis*

1. Introduction

Mexico is one of the centers of origin of the wild grapevines (*Vitis* spp.) For this country, at the end of last century, nearly 20 species were reported; and more recently, presence of most of those previously reported species has been confirmed (Table 1).

A: CONAFRUT, 1973; B: Comeaux, 1987; C: Comeaux 1991; D: Franco-Mora *et al.*, 2012; E: Martínez *et al.*, 2007; F: Rzedowsky and Calderon, 2005; G: Jaques and Salazar, 2012 (unpublished); H: Barber *et al.*, 2001; I: Van Zyl *et al.*, 2014; J: Remaily, 1987; K: Wiersma & Leon, 1999; L: Riaz *et al.*, 2007; M: Blanckaert *et al.*, 2004; N: Andersen *et al.*, 2010; O: López-Sandoval *et al.*, 2010; P: La Torre-Cuadros & Islebe, 2003; Q: León & Domínguez, 1989; R: Ibarra-Manríquez *et al.*, 1997.

From data of Franco *et al.*, 2012; MEXU: Herbario de la Universidad Nacional Autónoma de México; XAL: Herbario del Instituto Nacional de Ecología (Xalapa); CODAGEM: Herbario de la Universidad Autónoma del Estado de México; ENCB: Herbario del Instituto Politécnico Nacional; BUAP: Herbario de la Benemérita Universidad Autónoma de Puebla; CHAPA: Herbario del Colegio de Postgraduados; OAX: Herbarios del Instituto Politécnico Nacional en Oaxaca.

Species	Befo re 2000	After 2000	In herbariums¹
<i>V. acerifolia</i> Raf.			CODAGEM
<i>V. aestivalis</i> Michx.			CODAGEM
<i>V. arizonica</i> Engelm.	A; K	G; I; L	MEXU; ENCB; CHAPA
<i>V. berlandieri</i> Planch.	A	G	XAL; ENCB; BUAP; CHAPA
<i>V. biformis</i> Rose	A		MEXU; ENCB;
<i>V. blancoi</i> Munson	A		
<i>V. bloodwothiana</i> Comeaux	C		MEXU
<i>V. bourgaeana</i> Planch	A	H	MEXU; XAL; ENCB; BUAP; CHAPA; OAX
<i>V. caribaea</i> D. C.	A		MEXU; XALU
<i>V. cinerea</i> Engelm.	A	D; G; O	MEXU; XAL; CODAGEM; ENCB; CHAPA
<i>V. girdiana</i> Munson	K		MEXU; ENCB
<i>V. incisa</i> Jacq.		G	
<i>V. indica</i> Shwartz	A		
<i>V. indivisa</i> Willd.			ENCB
<i>V. jaegeriana</i> Comeaux	C		
<i>V. labrusca</i> L.	A		
<i>V. latifolia</i> H. et B.	A		ENCB; OAX
<i>V. lincecumii</i> Buckl.			CHAPA
<i>V. monticola</i> Buckl.			MEXU
<i>V. mustangensis</i> Buckl.		G	MEXU; CODAGEM; ENCB
<i>V. nesbittiana</i> Comeaux	B		
<i>V. peninsularis</i> Jones	A; Q		MEXU; ENCB
<i>V. popenoie</i> Fennel	A	F; G	MEXU; XAL
<i>V. riparia</i> Michx.	J		MEXU; CODAGEM; CHAPA
<i>V. rotundifolia</i> Michx.	A	N	MEXU; ENCB; CHAPA
<i>V. rupestris</i> Scheele	A		
<i>V. tiliifolia</i> H. et B.	A	E; F; G; P; R	MEXU; CODAGEM; ENCB; BUAP; CHAPA; OAX; XALU
<i>V. tuberosa</i> D.C.		M	ENCB

-V. incisa Jacq.		G	
V. indica Shwartz	A		
V. indivisa Willd.			ENCB
V. jaegeriana Comeaux	C		
V. labrusca L.	A		
V. latifolia H. et B.	A		ENCB; OAX
V. lincecumii Buckl.			CHAPA
V. monticola Buckl.			MEXU
V. mustangensis Buckl.		G	MEXU; CODAGEM; ENCB
V. nesbittiana Comeaux	B		
V. peninsularis Jones	A; Q		MEXU; ENCB
V. popenoie Fennel	A	F; G	MEXU; XAL
V. riparia Michx.	J		MEXU; CODAGEM; CHAPA
V. rotundifolia Michx.	A	N	MEXU; ENCB; CHAPA
V. rupestris Scheele	A		
V. tiliifolia H. et B.	A	E; F; G; P; R	MEXU; CODAGEM; ENCB; BUAP; CHAPA; OAX: XALU
V. tuberosa D.C.		M	ENCB

Table 1. Species of *Vitis* historically reported in Mexico

Nevertheless, changes in the use of land, constructions related with urban progress such as railways, residential zones, establishment of factories and others, reduced the space where naturally *Vitis* species grow in Mexico (Franco and Cruz 2012). Moreover, there is a lack of knowledge on the actual presence of each particular species of this genus because the difficulty of their correct species determination (Moore 1991). However, recently, *V. popenoie* was stated as in danger of extinction (Rzedowski and Calderón 2005) at least in the Bajío region in Central-West Mexico.

A national effort to conserve native *Vitis* species was initiated in the early 2000's (Franco *et al.* 2012). Nowadays, for Mexican wild species of this genus, it is established a national germplasm bank in Coatepec Harinas, Mexico (Bernal *et al.* 2012); and at least three local *Vitis* collections are reported in the states of Puebla, Veracruz and Mexico (Franco *et al.* 2012). From 2005, the states where the *Vitis* plants were GPS positioned included Puebla, Mexico, Veracruz, Morelos, Nayarit, Tabasco, San Luis Potosí, Hidalgo, Guerrero y Michoacán (Franco-Mora *et al.* 2008; Cruz-Castillo *et al.* 2009; Tobar-Reyes *et al.* 2009). In this sense, some studies on taxonomy, floral biology, reproduction, contents of biological compounds, agro-industrial and culinary uses, as well as agricultural practices have been conducted (Tobar-Reyes *et al.* 2009; Tobar-Reyes *et al.* 2011; Aguirre *et al.* 2012; Jiménez *et al.* 2012; Franco-Mora *et al.* 2012; Salomón *et al.* 2012; Jimenez-Martínez *et al.* 2013; Franco-Mora *et al.* 2016). Breeding of *V. vinifera*, one of the most cultivated fruit crop in the world (Londo and Martinson 2015) implies the conservation of American *Vitis*, including those native of Mexico. Some American *Vitis* are resistance-tolerant to phylloxera (*Daktulosphaira vitifoliae* Fitch) (Downie *et al.* 2000); presence of nematodes (Van Zyl *et al.* 2014) limey soils (Grundler *et al.* 2015), and they might be an interesting genetic resource to help solving the forthcoming queries in *V. vinifera* cultivation.

On the other hand, the berries of Central Mexican wild grapevines are usually small and unsweet. During 3 continuous years, it was determined an average of 0.404 g per berry and a total soluble solid content of 16.9°B in grapes of *V. cinerea* growing in the South of the State of Mexico, Mexico (Franco-Mora *et al.* 2012). Thus, although human consume of those berries is reported, it is possible that the development of alternative uses for the berries of wild grapevines will increase the interest of locals for preserving this plant genetic resource. One potential use is obtaining seed oil; the seeds of grapes growing in situ and ex situ presented over 16% (w/w) oil. In that oil, four fatty acids were determined, linoleic, oleic, palmitic and stearic acids (Franco-Mora *et al.* 2015)

In present report, the scheme for conservation and utilization of wild grapevines of the States of Puebla, Mexico and Morelos, in Central Mexico, is presented.

2. Materials and Methods

Location of wild grapevines

Several trips to locate wild grapevines were performed in the States of Puebla, México and Morelos. Their locations were recorded with a GPS, and then, pointed out in a map with the free software DIVA-GIS. When possible, some cuttings were taken and then, under greenhouse conditions, induced to root (Jiménez *et al.* 2013)]. Successfully obtained plants were donated to the National Vitis Germplasm Bank (Bernal *et al.* 2012) and for some accessions their copy or copies were kept for the members of the Vitis group for further studies.

Cropping

Nowadays, in the Working Vitis Collection in Zumpahuacán, Mexico, there are planted 76 accessions from the states of Puebla, Mexico and Morelos (Fig. 1). The distance from plant to plant is 50 cm and between lines 80 cm. The vines were pruned and fertilized with lombricompost tea and a commercial fertilizer, 15-15-15 or nitrofoska ®. Control of pest and diseases has been done by applying recommendations for *V. vinifera*.

Fruit quality and yield characterization

Since 2013, berry weight, height and width, and the content of total soluble solids had been determined in 23 grapevines. As berry sweetness and size are important components for selection and breeding (Shiraish and Shiraishi 1997), a figure relating both fruit characteristic was arranged. In 2015, because production has become stable, the yield of each plant has been recorded and those plants with higher production have been selected to increase the plant number and start a small plantation of cloned grapevines.

Fatty acid in seed oil

In 2014, we extracted, with hexane (Franco-Mora *et al.* 2015), the oil in seeds of accession Puebla-78 (P-78), a mix of seeds from the plants native of the State of Mexico and a mix of seeds of plants native of the State of Puebla. In 2015, the seed oil was extracted from accessions Morelos-45 (Mo-45), P-86, P-78, P-178 and EdoMex-150 (E-150). Then, those samples were sent to a certified laboratory to their fatty acid determination (Franco-Mora *et al.* 2016).

3. Results

Plant location

The states of Puebla, Mexico and Morelos, located in Central Mexico, have regions where the wild grapevines grow extensively (Fig. 1).

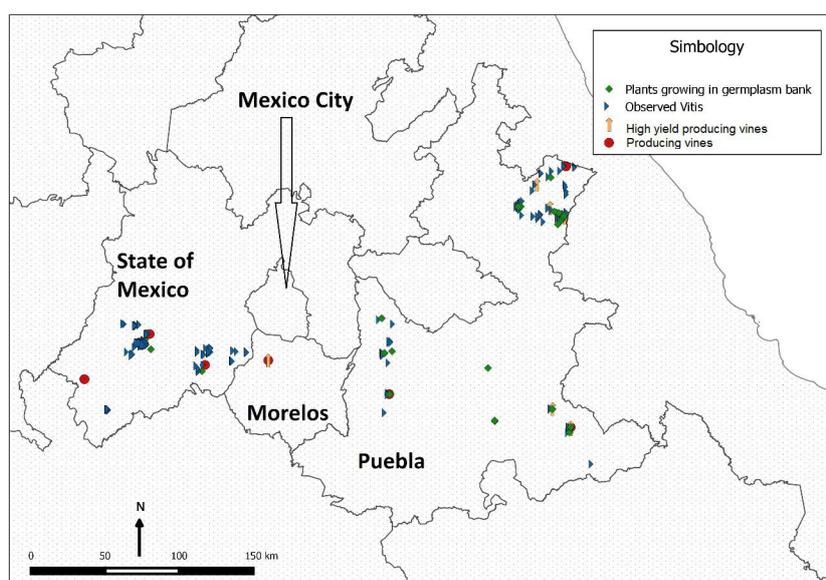


Figure 1. Map of Central Mexico showing the places of location of wild grapevines (*Vitis* spp.), pointing out the location of

plants than have fruited, fruiting continuously in three years and with higher fruit production in 2015.

In the working collection in Zumpahuacán, Mexico, it has been determined the presence of plants of *V. cinerea* (López-Sandoval *et al.* 2010; Franco-Mora *et al.* 2012), *V. popenoie* and *V. mustangensis* (Fig. 2). The rest of the accessions are under further analyzes for correct species determination.



Figure 2. Fruits of *Vitis popenoie* (left) and leaves and flowers of *Vitis mustangensis* (right) in vines growing ex situ in Zumpahuacán, Mexico.

Fruit size and TSS content

Most of the berries weighted less than 0.5 g, and 90% of the fruit were lower than 1 g. Fruit of accessions P-41 (2013) and P-64 (2015) were over 2.0 g (Fig. 3). Whereas most of the berries have TSS between 14 and 18°B, 4 accessions presented values between 20 and 22°B. By dividing the accessions according to classification of Shiraishi and Shiraishi (1997) for fruit weight and TSS content, only those berries of P-18 harvested in 2013 were over 1 g and had 18°B.

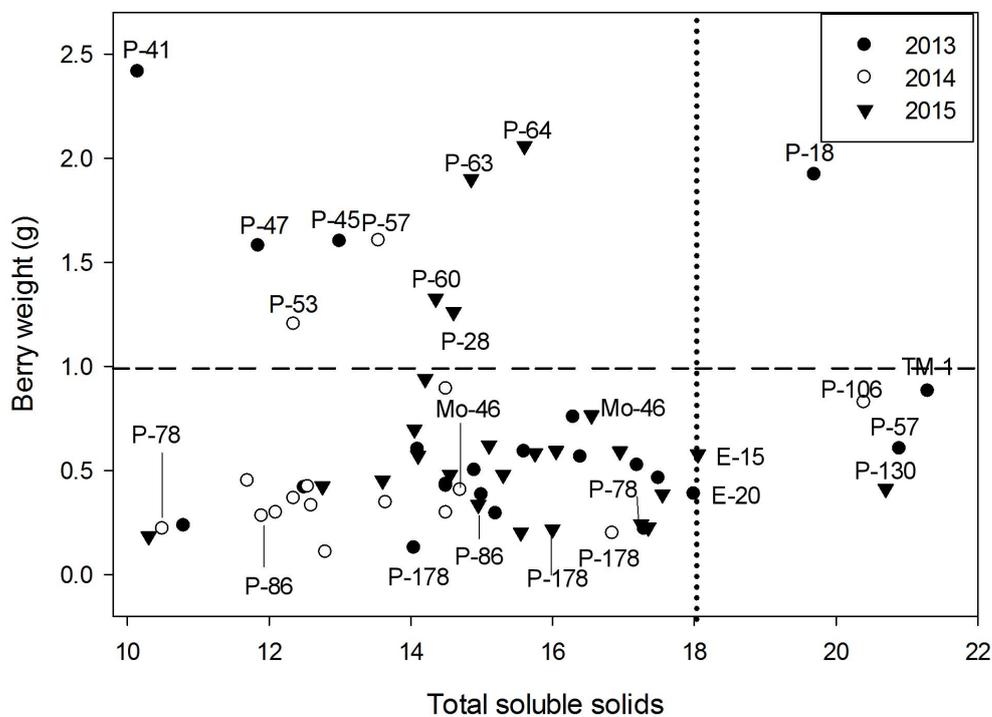


Figure 3. Fruit weight and total soluble solid content in the berries of *Vitis* spp. growing in Zumpahuacán, Mexico in 2013, 2014 and 2015.

In 2015, three accessions have fruited over one kilogram per plant (Table 2, Fig. 4), and under present conditions of cropping, it has been estimated a production over 60 t ha⁻¹ for the accession P-78, native of Hueytamalco, Puebla.

Table 2. Berry production in 2015 in four Vitis accessions growing in Zumpahuacán, Mexico.

Accession	Yield per plant (kg)	Estimated production* (t ha ⁻¹)
P-78	2.500	62.0
P-178	1.500	37.5
P-86	1.400	35.0
Mo-46	0.680	17.0

* Estimation is based on a density of 25000 plants per hectare, actual conditions of the germplasm bank.



Figure 4. Racimes of the accessions Puebla-178 (P-178) (left) and Puebla-78 (P-78) (right) in 2015 in Zumpahuacán, Mexico.

Seed oil

There were determined four main fatty acids (Fig. 5 and 6) in the oil of Vitis seeds. Within those four acids, e.g. linoleic, oleic, palmitic and stearic, all were found in the respective oil of 8 samples, with exception of the stearic acid, absent only in the oil of EdoMex (2014). Others fatty acid present in some of the oils in low levels were linolenic acid, behenic acid, eicosapentanoic acid, palmitoleic acid, arachidic acid, elaidic acid, linolelaidic acid and lignoceric acid.

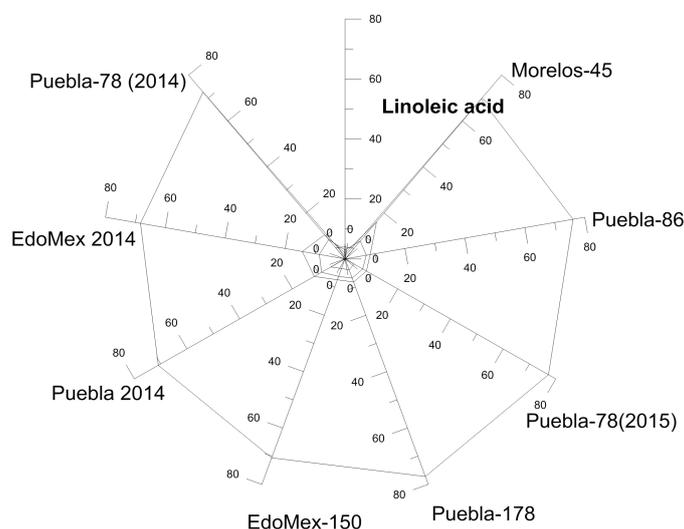


Figure 5. Linoleic acid content in the seed oil of 8 Vitis growing in a germplasm bank in the South of the State of Mexico.

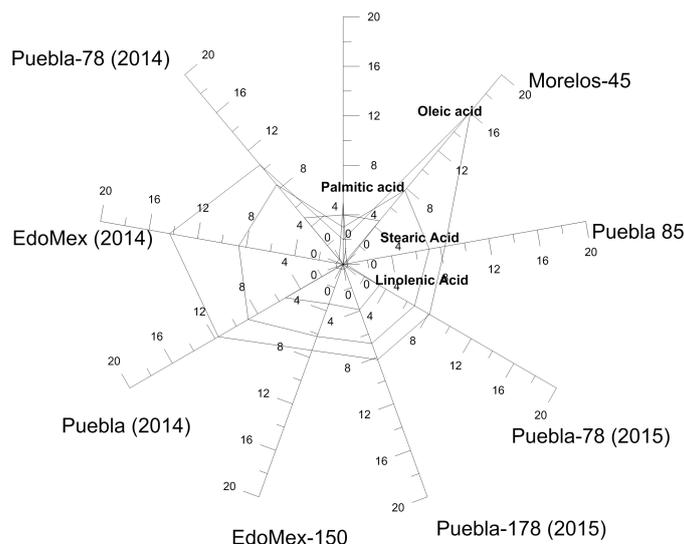


Figure 6. Oleic, palmitic, stearic and linolenic acid contents in the seed oil of 8 *Vitis* growing in a germplasm bank in the South of the State of Mexico.

4. Discussion

In the states of Puebla, Mexico and Morelos, in Central Mexico, there are several regions where native populations of wild grapevines grow. It has been determined a huge vegetative and fruit quality diversity among *Vitis* native of the same region (Franco-Mora *et al.* 2008; Cruz-Castillo *et al.* 2009; Franco-Mora *et al.* 2012). In this sense, conservation of all the variability is nearly impossible due to the high requirements of time, money and staff. Breeding of *V. vinifera* for fruit production has been performed regarding in fruit size and sweetness (Alleweldt and Possingham 1988). According to those parameters, in present work, the fruit of accessions P-41 (Teziutlán); P-64 (Juan N. Mendez), P-63 (San Antonio Cañadas) and P-18 (Teziutlán) presented the higher values for fruit weight, between 1.8 and 2.4 g per berry. Those grapes and those fruited by P-45, P-47 (Teziutlán), P-53, P-57, P-60 (San Antonio Cañadas) and P-28 (Chapulco) are classified as small berries; whereas the rest of the harvested berries were classified as very small ones (Shiraishi and Shiraishi 1997). Fruits with very small size are typical in several species of *Vitis* native of America and particularly Mexico (Shiraishi and Shiraishi 1997; Franco-Mora *et al.* 2012). The grapevines from Teziutlán and San Antonio Cañadas, Puebla State, seem to be well adapted to the conditions of Zumpahuacán.

Higher fruit size, was positively correlated with TSS only for berries of P-18; whereas the rest of the berries belonging to the sweetness category 7 (between 20.1 and 24°B) (Shiraishi and Shiraishi 1997) presented very small fruit. For *V. vinifera* some researchers have determined that fruit sweetness is related to fruit size (Ferrer 2014), however as most of wild grapevines in Mexico had not been bred, fruit quality has not been improved for human consume.

The grapevines producing more yield fruit in 2015 were originally collected in Teziutlán (P-78), Hueytamalco (P-178) and Cuetzalán (P-86), Puebla; and Cuernavaca (Mo-46), Morelos. For present grapevine collection, it is interesting, that the grapevines which were originally collected in the State of Mexico had not a high yield potential, at less when growing in a place of the same state, implying similar environmental conditions. Contrarily, Teziutlán Region, in Puebla, appears as an interesting place to continue collecting grapevines, which might be productive, and also some of them might get interesting sizes and contents of TSS when growing in the State of Mexico, specifically in Zumpahuacán.

It has been determined that the seed oil of wild Central Mexico *Vitis* is closely similar to the quality of *V. vinifera* oil (Franco-Mora *et al.* 2015). The results of present report agreed, as the main fatty acid in all the analyzed oils is linoleic acid, between 68 and 78% (w/w). Nevertheless, some differences, possible related to genetic or environmental conditions were observed. For example, in the oil of mix of seeds from the accessions of the State of Mexico (2014) it

was not present the stearic acid; whereas recently, in the seed oil of three samples from the State of Mexico that acid was reported (Franco-Mora *et al.* 2015). In that former report, only the four main fatty acids observed in present research, linoleic, oleic, palmitic and stearic acids, were determined. For this paper, with a more detailed methodology, few amounts of other 8 fatty acids were observed in the seed oil of wild grapes, that is, linolenic acid, behenic acid, eicosapentanoic acid, palmitoleic acid, arachidic acid, elaidic acid, linolelaidic acid and lignoceric acid. Those fatty acids were reported to be present, also in low amounts, in *V. vinifera* seed oil (Fernandes *et al.* 2013). The seed oil analysis confirms that one potential use of this Central Mexico *Vitis* species is the extraction of seed oil. In this sense, as the seed weight represents an important percentage of the total fruit weight (Franco-Mora *et al.* 2012) its useful management might improve the interest in conserving this plant genetic resource.

Conclusions

Fruit weight of most of Central Mexico wild grapevines growing in Zumpahuacán, Mexico was below 1 g; and the content of total soluble solids was between 10 and 18°B. In the seed oil the main fatty acid was linoleic acid with a range content between 68 and 78%; other fatty acids representative present were oleic, palmitic and stearic; additionally few amounts of linolenic acid, behenic acid, eicosapentanoic acid, palmitoleic acid, arachidic acid, elaidic acid, linolelaidic acid and lignoceric acid were observed.

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Compliance with ethical standards

Conflict of interest

The authors declare that they do not have conflict of interest.

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