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SHORT COMMUNICATION

Exploring genetic variation, oil and α-tocopherol content in avocado (*Persea americana*) from northwestern Mexico

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Abstract The Hass cultivar of avocado is the most widely grown commercial cultivar in Mexico. Unfortunately, this cultivar is poorly adapted to the Mexican low-lands with hot dry climates characteristic of northwestern Mexico. Other well-adapted avocado accessions are available for these regions, but their nutritional traits and genetic diversity have yet to be explored. In this study, we analyze oil content, (α-tocopherol) and genetic variation among five local varieties from northwest Mexico that grow in high temperatures regimes in unfertilized soils and without any agronomic management. We report significant phenotypic variability in oil and α-tocopherol components in different accessions of avocado as determined by HPLC. Interestingly, we find higher α-tocopherol content (45.02–50.66 μg/g of fresh pulp) in some local avocados compared to Hass (32.28 μg/g of fresh pulp). The analyzed accessions represent a moderately polymorphic set of genotypes as measured by microsatellite (10 alleles by locus) and SNP (1 SNP every 164.4 bp) analysis of the VTE3 and VTE4 genes, implied in the biosynthesis of tocopherols. SNP data allowed also identifying differences between the local varieties and controls (Hass and the Mexican race accession). The variation observed at the genetic, morphologic and nutritional levels provide significant new information that may be valuable in selecting and developing avocado genotypes adapted to high-temperature environments.

Keywords α-Tocopherol · Genetic diversity · Local germplasm · Oil · *Persea americana*

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Introduction

Avocado (*Persea americana* Mill.) is a highly nutritive and economically important fruit. México is the main producer and exporter (SIAP 2015) of avocado where commercial production is concentrated in temperate areas (e.g. Michoacan). Hass, the most widely grown commercial cultivar, does

not grow well in arid, low elevation regions of Mexico, where commercial production is quite limited. But, local genotypes are grown in small scale orchards and family gardens in arid regions and there is abundant local avocado germplasm that may provide a valuable source for selecting and developing new varieties adapted to high temperature regimes.

Avocado is an excellent source of monosaturated fatty acids, fiber, protein, vitamin E, and phytochemicals with great biological value, such as phytosterols and phenolic compounds, (Rodríguez-Carpena et al. 2011; Dreher and Davenport 2013). These compounds, and especially vitamin E, have been related to protection against oxidative stress and have anti-inflammatory properties (Shirpoor et al. 2016). Wide phenotypic variation in nutrient-related traits has been studied before, in particular, for carotenoids, β-sitosterol, α-tocopherol and fatty acid concentration in fruit pulp (Lu et al. 2009; Calderón-Vázquez et al. 2013). Moreover, quantitative genetic analyses have established that variation in nutrient content is predominantly genetically determined (Calderón-Vázquez et al. 2013) opening the possibility of further nutritional enhancements through breeding.

Cultivable soils of Northwest México are dominated mainly by a BSh (arid hot steppe) climate according to the Köppen–Geiger scale (Peel et al. 2007) with warm to hot summers, however, the lowland areas are agroecologically suitable for growing some local avocado genotypes. These are cultivated mainly in family gardens or small orchards with little or no agronomic management and may be valuable to the local communities as a source of vitamin E and other essential nutrients. Its morphology suggests that these are different from Hass variety or the Mexican race. Beyond the high morphological variation observed in local avocados, genetic variation, nutritional composition and breeding potential of local genotypes remains largely unexplored. Characterizing existing variation is a necessary first step to determine the potential of local genotypes as a source of adaptive traits for the expansion of commercial avocado cultivation into new climatic regions. This study reports nutritional (oil and α-tocopherol) characteristics assessed along with a genetic evaluation of avocado accessions from Guasave, Sinaloa at Northwestern México.

Materials and methods

Based on differences of fruit morphology, five contrasting (and locally consumed) avocado accessions from Guasave, Sinaloa were collected; a Mexican race accession (Pequeño) and Hass were used as controls (Online Resource 1). Five mature fruits of each genotype were evaluated for fresh and dry weight of whole fruit, pulp, seed and skin. The pulp was freeze-dried and stored at −20 °C.

Oil extraction and α-tocopherol analysis

Oil was obtained with Soxhlet extraction (Lee et al., 1983) using 0.5 g of avocado flour and 70 mL of hexane with 0.01% of butyl-hydroxytoluene (BHT, Sigma-Aldrich, St. Louis, MO, USA). α-tocopherol was extracted according to Cerretani et al. (2010) with some modifications. Briefly, 400 µL of oil was extracted twice with 1 mL of absolute methanol containing 0.1% BHT, and once again with 1 mL of methanol:isopropanol (80:20). Extractions were performed for 30 s with shaking, centrifuged at 8000*g* for 10 min and then evaporated to dryness using a rotary evaporator at 37 °C. The resulting residue was suspended in 1 mL of methanol. Three extracts were obtained per sample and three independent experiments were carried out.

The determination of α-tocopherol was done in a DIONEX Ultimate 3000 HPLC system with a diode array detector; injecting 10 µL of sample diluted 1:20 in acetonitrile:methanol:water (75:8:17). The mobile phase consisted of: (A) 0.015% formic acid in water, (B) 17/83 methanol/acetonitrile. The following separation gradient was used: 100% A for 3 min, 0–45% B in 5 min, 45–100% B in 0.1 min, 100% B for 21.9 min, and 100% A for the last 10 min. All separations used a flow rate of 0.25 mL/min and 293 nm allowed detection of the vitamin.

Genetic analysis

Total genomic DNA was isolated from young leaves using 3% cetyltrimethyl ammonium bromide (CTAB) according to Doyle and Doyle (1987). Individuals were genotyped at five highly polymorphic microsatellite loci (AVD.001, AVD.006, AVD013, AVD.022, and AVO.102) as previously described by

Ashworth and Clegg (2003). PCR products were separated by capillary electrophoresis on the 3500 genetic analyzer (Thermo Scientific). Microsatellite alleles were visualized and scored in GeneMarker (SoftGenetics, PA USA). The total number of alleles, polymorphic index content (PIC), and observed and expected heterozygosities were calculated by using FSTAT software V 2.9.3.2 (Goudet 2001).

Genetic relations among individuals were evaluated by sequencing fragments of the VTE3 and VTE4 genes implied in the biosynthesis of tocopherols (Lushchack and Semchuk 2012). Primer sequences are shown in the Online Resources 2. Gene fragments were amplified on a C1000 Touch (BIORAD) thermal cycler (3 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 30 s at 57.3 °C and 1 min at 68 °C, and a final cycle of extension at 68 °C for 5 min), purified and sequenced. Sequences were aligned using codon code aligner (CodonCode, Dedham, MA, USA). Posteriorly, fragments were concatenated and analyzed as a unique sequence in MEGA 6 (Tamura et al. 2013). Genetic variation among individuals was evaluated through the index of nucleotide diversity (π) and single nucleotide polymorphism.

Results and discussion

Morphology and pulp composition

Significant differences in fruit weight were found between accessions (Online Resource 1). Proportions of seed, pulp and skin were similar among all accessions; which could be attractive for further commercialization. Dry weight in Guasave avocado ranged from 15.1 to 23.01% (Fig. 1a). These values are significantly lower than the observed for Hass and Pequeño with 26.1 and 39.4%, respectively.

Percentage of oil content ranged from 7.39 to 36.98%. Pequeño and Hass showed higher concentrations than local Guasave avocados (Fig. 1b). Oil percentages of our local samples are comparable or higher than previously reported for genotypes of the West Indian race (Gómez-López 2000, 2002). Variation in total lipid content between fruits might be associated with maturity stage but also with environmental conditions, postharvest management, geographical conditions and genetic background (Wedding et al. 2013; Hurtado-Fernández et al. 2014; Yu et al. 2016).

Interestingly, we found higher concentrations of α-tocopherol in Palma, Pequeño and Gve8 accessions (50.66, 47.63, 45.02 μg/g of fresh pulp, respectively) compared to Hass (32.28 μg/g of fresh pulp) (Figure 1C). The USDA database (2015) reported tocopherol contents of 19.7 and 26.6 μg/g in avocado from California (Hass) and from Florida, respectively, but does not specify the genetic background of Florida fruits. Further research is needed to understand whether differences in oil and tocopherol found for the analyzed accessions are due to its racial origin or to environmental conditions. Lu et al. (2009) reported α-tocopherol contents of 16.27 to 27.57 μg/g in Hass fruits cultivated in 4 different locations and harvested during four different harvest seasons in a single year. They suggest that these variations depend mainly on the harvest season showing the highest values during the last part of the year among all studied locations. Despite the fact that this vitamin is lipophilic, we did not observe any correlation between α-tocopherol with oil content or dry matter ($r = 0.3358$ and $r = 0.4391$, respectively, based on a Pearsońs correlation coefficient). Stevenson et al. (2007) in pumpkin seeds and Rani et al. (2007) in soybean also did not find correlations between these parameters. Overall, our results indicate that avocado accessions from Guasave, Sinaloa have nutritional characteristics comparable or superior to avocados from other growing regions around the world, including temperate regions of Mexico and California and point to the potential of the avocado from this agroclimatic area as a source of bioactive compounds.

Genetic data

The five-microsatellite loci analyzed showed high levels of genetic diversity with 10 alleles per locus and PIC values averaging 0.62 (Table 1). This represents a high genetic diversity level but comparable with previous reports in avocado (Gross-German and Viruel 2013; Abraham and Takrama 2014) using microsatellite markers. Microsatellite loci showed that local accessions have 5.8 alleles per individual, compared with 8.5 alleles observed in the control genotypes. We were unable to find any relation between genetic diversity, avocado race or accession. Even though local avocados are geographically separated from Hass and Pequeño, they share some alleles, which suggests partial but not complete

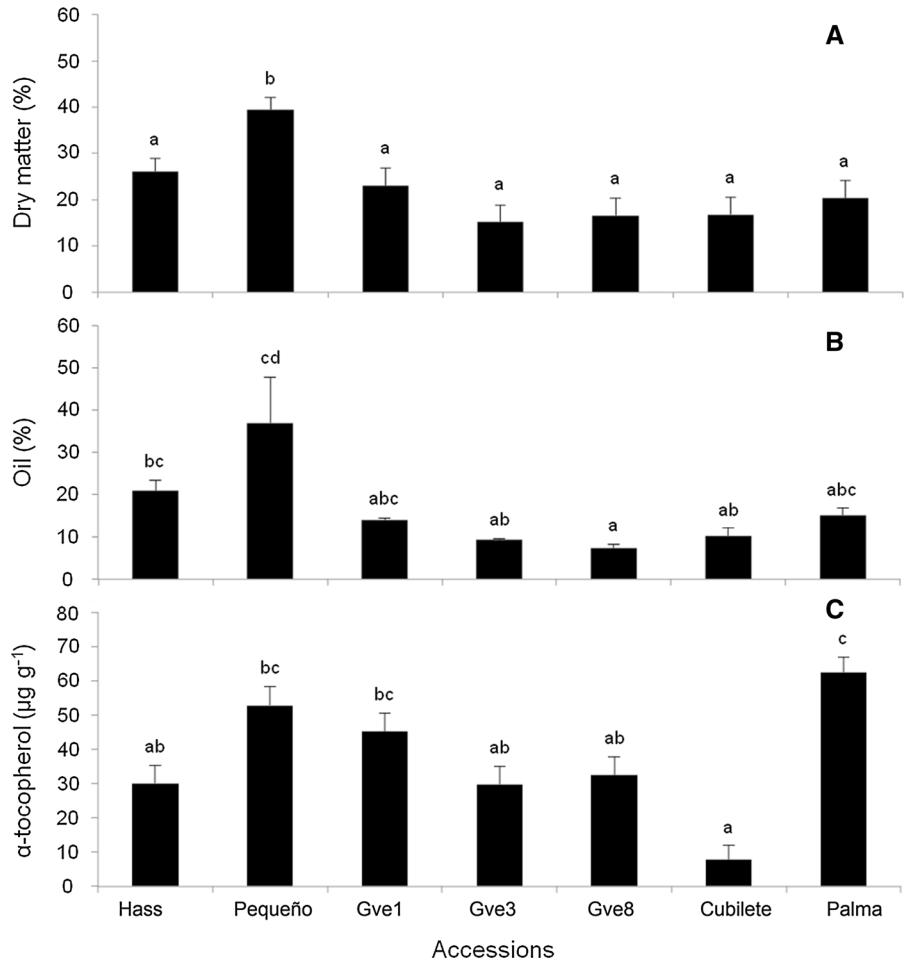

Fig. 1 Percentage of dry matter (**a**), percentage of oil (**b**) and contents of α-tocopherol (μg/g) (**c**) of avocado accessions. Data are the means of three biological replicates and three independent experiments plus SD. *Different letters* means statistically significant difference between accessions ($p \leq 0.05$)

Table 1 Genetic diversity in terms of A, number of Alleles; HO–HE, observed and expected heterozygosity and PIC, polymorphic index content of the avocado accessions

Accession	A	HO	HE	PIC
Hass	9	0.8	0.8	0.30
Pequeno	8	0.6	0.6	0.22
Gve1	6	0.2	0.1	0.07
Gve3	8	0.6	0.6	0.22
Gve8	8	0.6	0.6	0.22
Cubilete	7	0.4	0.4	0.15
Palma	7	0.4	0.4	0.15

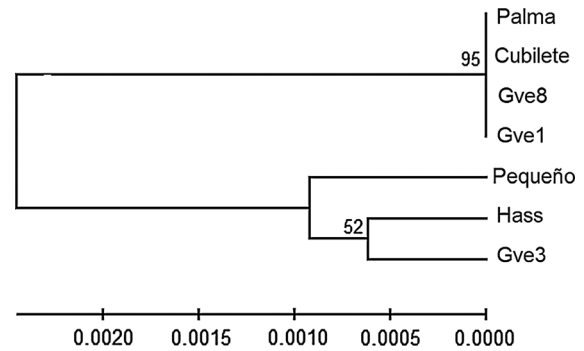

Fig. 2 UPGMA dendrogram showing the genetic relation among avocado accessions from Guasave Sinaloa, Mexico

genetic differentiation between the Guasave and control genotypes.

Sequence data from the two SNP markers, VTE3 and VTE4, yielded in 822 bp of aligned sequence. This sequence was used to construct a dendrogram that depicts genetic relationships among accessions (Fig. 2). Similar to the microsatellite data, the sequence analysis showed lower genetic diversity in Guasave accessions, compared with the controls. Excepting GVE3, Guasave avocados show little

Table 2 Polymorphic sites and SNPs detected on the sequence of the VTE3 and VTE4 genes

	VTE3 (440 bp)						VTE4 (382 bp)										
Position	31	58	85	235	358	370	24	121	138	164	189	213	215	227	248	294	318
Consensus	G	**A**	C	G	**T**	**G**	C	**A**	G	C	C	A	C	C	C	**C**	T
Hass	.	**G**	.	.	**C**	.	.	**C**	.	.	.	.	.	.	.	Y	.
Pequeno	.	.	Y	.	**C**	.	M	**C**	.	.	.	M	.	.	.	.	.
Gve1	.	.	.	.	.	**A**	.	M	.	.	.	.	.	.	.	.	.
Gve3	.	**G**	.	R	**C**	.	M	**C**	K	M	M	M	Y	M	M	**T**	Y
Gve8	K	.	.	.	.	**A**	.	.	.	.	.	.	.	.	.	.	.
Cubilete	.	.	.	.	.	**A**	.	M	.	.	.	.	.	.	.	.	.
Palma	.	.	.	.	.	R	.	.	.	.	.	.	.	.	.	.	.

Shaded letters are SNP and non-shaded letters heterozygotes at IUPAC code

genetic differentiation so that they are clustered into the same group but separated from Hass and Pequeño. Observed heterozygosity ranged from 57 to 71% in VTE3 and VTE4, respectively. These values are relatively high but comparable with those previously reported by Chen et al. (2008, 2009) at four loci, whose values varied from 40 to 70% in avocados from different wild races and commercial cultivars.

On the other hand, the analysis of nucleotide diversity showed lower polymorphism levels (1 SNP every 164.4 bp; $\pi = 2.89 \times 10^{-3}$) in the Guasave accessions compared with (1 SNP every 33.7 bp; $\pi = 6.58 \times 10^{-3}$) values reported by Chen et al. (2008, 2009). This lower genetic diversity might be due to differences in polymorphism in the specific genomic locations analyzed, or it may also result from a more limited geographic and genomic sampling effort. Chen et al. sampled individuals from different localities and from different racial origin of avocados from Mexico, Costa Rica, Ecuador and Dominican Republic. Furthermore, they analyzed four loci that resulted in 5960 bp of the genome. In this study, we analyzed individuals from a specific region of the State of Sinaloa in Mexico (Online Resources 3) for only two loci that resulted in 822 bp of the genome. The VTE3 gene showed three SNPs and three heterozygotes. The VTE4 showed only two SNPs and nine heterozygotes, all of them were present in the GVE3 accession, which suggests a possible hybrid origin for this accession (Table 2), however, more research is required to support this hypothesis. Interestingly GVE3 shared three SNPs with Hass, which has been reported as a hybrid between G × M races (Chen et al. 2009). GVE3 showed the highest genetic variation with seven heterozygotes and five SNPs followed by Hass with one heterozygote and four SNPs.

Conclusions

Trees with large fruits and a large mesocarp/seed ratio were identified in Guasave, Sinola and these could be favorable for pulp production. Compared to Hass, some Guasave fruits show high α-tocopherol values. Moderate allelic variation was also found at the SSR and SNP levels. SNP located in the VTE3 and VTE4 genes also allowed separating controls (Hass and Pequeño) from local accessions. Although other agronomic properties of the genotypes must be evaluated, i.e. fruit yield; the evaluated Guasave

genotypes show promise for breeding programs aimed at the production of heat-tolerant genotypes with high nutritional value. Ultimately, this could open the possibility for the expansion of commercial avocado production into hot and arid regions of Mexico.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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