

Identification of morphometric traits for screening of tejocote (*Crataegus* spp.) germplasm for better yield potential

Carlos A. NÚÑEZ-COLÍN^{1*}, Jaime SAHAGÚN-CASTELLANOS², Fernando GONZÁLEZ-ANDRÉS³, Alejandro F. BARRIENTOS-PRIEGO², Sergio SEGURA⁴, Raúl NIETO-ÁNGEL²

¹ Campo Exp. Bajío, Inst. Nac. Investig. For., Agric. Pecu., km 6.5, Carret. Celaya-San Miguel de Allende, Celaya, Guanajuato, 38110 Mexico

² Inst. Hortic., Dep. Fitotec., Univ. Autón. Chapingo, km. 38.5, Carret. México-Texcoco, Chapingo, 56230, State Mex., Mexico
lit007a@gmail.com

³ Dep. Ing. Cienc. Agrar., EST Ing. Agrar., Univ. León, Ave. Portugal 41, León, 24071, Castilla y León, Spain

⁴ Cent. Reg. Univ. Cent. Occidente, Univ. Autón. Chapingo, Ave. Perif. Indep. 1000, Morelia, 58170, Michoac., Mexico

Identification of morphometric traits for screening of tejocote (*Crataegus* spp.) germplasm for better yield potential.

Abstract — Introduction. Tejocote (*Crataegus* spp.) is an edible fruit species that some Mesoamerican cultures have cultivated since pre-Hispanic times in Mexico; however, this fruit crop has not undergone formal breeding. It is unknown whether morphological markers associated with fruit size exist to be used for indirect selection; this could be important, because this species has a large cycle. Our research aimed at studying phenotypic correlations between leaf and stomata characters with fruit weight; such correlations might be used as morphological markers for selection of superior germplasm for fruit weight. **Materials and methods.** Pearson's correlation coefficients of 36 leaf variables and three leaf stomata variables with fruit weight were calculated in 94 genotypes of four different species. **Results.** As expected, the correlations calculated for each species and for the species pool differed. However, the number of veins in leaves of reproductive shoots was positively correlated with fruit weight ($P \leq 0.05$) in the two most important horticultural species. On the other hand, *C. mexicana* was the best option for selecting superior germplasm, and the most significant variables correlated with fruit weight in this species were leaf major axis length of large vegetative shoots, leaf elongation ratio and number of veins of reproductive shoot leaves. **Discussion.** These variables could be good selection indices or morphological markers that can be used for early screening of germplasm for yield potential.

Mexico / *Crataegus* spp. / plant breeding / choice of species / selection criteria / fruits / yields

Identification de caractères morphologiques permettant la sélection de matériel génétique de tejocote (*Crataegus* spp.) pour l'obtention d'un rendement amélioré.

Résumé — Introduction. Le tejocote (*Crataegus* sp.) est une espèce à fruits comestibles cultivée par les Mésoaméricains depuis l'époque pré-hispanique au Mexique ; cependant, cette culture fruitière n'a pas fait l'objet d'une réelle sélection. On ne sait pas si des marqueurs morphologiques associés à la dimension de fruit existent ; ils pourraient être utilisés pour une sélection indirecte, ce qui serait intéressant du fait du long cycle de production de l'espèce. Nous avons cherché à étudier des corrélations phénotypiques entre certains caractères de la feuille et des stomates et le poids de fruit ; de telles corrélations pourraient être utilisées comme marqueurs morphologiques pour le choix de matériel génétique supérieur en ce qui concerne le poids du fruit. **Matériel et méthodes.** Des coefficients de corrélation de Pearson entre 36 variables de feuille et trois variables de stomates de feuille et le poids du fruit ont été calculés pour 94 génotypes appartenant à quatre espèces du genre *Crataegus* différentes. **Résultats.** Comme prévu, les corrélations calculées pour chaque l'espèce ont différé de celles obtenues pour les espèces considérées globalement. Cependant, le nombre de nervures des feuilles des rameaux reproducteurs a été significativement corrélé avec le poids de fruit ($P \leq 0,05$) dans les deux espèces les plus importantes en horticulture. Par ailleurs, *C. mexicana* s'est révélée la meilleure espèce pour sélectionner du matériel génétique de qualité et, pour cette espèce, les variables les plus importantes corrélées avec le poids de fruit ont été la longueur de l'axe principal des feuilles des grands rameaux végétatifs, le rapport d'élongation [rapport (axe longitudinal / axe radial)] des feuilles et le nombre de nervures des feuilles des rameaux reproducteurs. **Discussion.** Ces variables pourraient être de bons index de sélection ou des marqueurs morphologiques pouvant être utilisés pour le criblage précoce du matériel génétique vis-à-vis des potentialités de rendement.

Mexique / *Crataegus* spp. / amélioration des plantes / choix des espèces / critère de sélection / fruits / rendement

* Correspondence and reprints

Received 16 May 2008
Accepted 21 July 2008

Fruits, 2009, vol. 64, p. 35–44
© 2009 Cirad/EDP Sciences
All rights reserved
DOI: 10.1051/fruits:2008048
www.fruits-journal.org

RESUMEN ESPAÑOL, p. 44

1. Introduction

Tejocote (*Crataegus* spp.) belongs to the Rosaceae family, Maloideae subfamily. Thirteen species have been identified from Northern and Central Mexico, and, from the South, two or more species may exist [1–3]. In Mexico, this genus is widely distributed, mainly in the Sierra Madre Oriental, the Neovolcanic axis and the highland of Chiapas, principally in cold and temperate weather, at altitudes between (400 and 3000) m above sea level [2, 4].

Tejocote has been cultivated and it has been the object of selection since pre-Hispanic times in Mexico. However, tejocote is a fruit crop species without formal breeding; it belongs to a genus whose taxonomy is complicated because of its many reproductive problems, such as high polyploidy (2x, 3x, 4x, 5x and 6x, with $x = 17$) [5], sterile male agamospermy, hybridism [6, 7], apomixis [7–9], pseudogamy and gametophytic self-incompatibility, as occurs in apple (*Malus x domestica* Borkh.) [10]. Besides, the time required for a breeding cycle that includes sexual reproduction, a juvenile period and evaluation of fruit yield may be too long for the plant breeder. Moreover, in some fruit species of the Rosaceae family, the leaf characters have been used as morphological markers associated with fruit yield characters [11, 12].

The objective of our research was to identify the leaf and stomata variables correlated with the fruit weight, and, in this way, to establish these variables as morphological markers to be used for indirect selection, collection and breeding programs to improve fruit yield.

2. Materials and methods

Our study was carried out with 94 accessions (*table I*) from the germplasm bank of tejocote (*Crataegus* spp.) located in the San Juan Experimental Field of the Universidad Autónoma Chapingo, Mexico, (lat. 19° 29' N, long. 98° 53' W, alt. 2240 m above sea level). The accessions represent individual clones collected as bud wood between 1982 and

1989, then grafted onto seedling rootstocks of tejocote. These accessions were established in triangular planting with 5 m between trees.

Thirty-six leaf variables and three stomata variables were used to identify morphological markers associated with fruit yield (*table II*) because these structures may allow making selection in both seedling plants and plants that still do not produce fruits.

The leaf variables were obtained by image analysis using the Image tool version 3.00 [13]. Twenty leaves of each shoot type without damage were collected from the middle part of the tree in sunny conditions from the four cardinal points of the tree. These leaves were scanned using a Genius Color Page Vivid Pro II Film scanner, and processed with the software PhotoImpact Version 4.2. In addition, stomata variables were obtained by the same image analysis, but the samples were prepared with dental silicon (Exactodent®) to obtain a negative image, and with transparent nail polish to obtain the positive image. The sample units were obtained from the middle part of the first young, but mature, leaf on the shoot. The measurements were made on 10 samples per preparation in a Leica Galen III microscope, with objective at 40× and ocular at 10×, adapted to a Leica ET-8800N digital camera and a GrabIT Pro image capture card for their digitalization.

Feret diameter (FD) of the leaves was calculated with the formula: $FD = \sqrt{[(4 \times \text{area}) / \pi]}$; elongation ratio (ER), with $ER = [\text{minor axis length} / \text{major axis length}]$; compactness ratio (CR), with $CR = [\text{feret diameter} / \text{major axis length}]$, and roundness ratio (RR), with $RR = [(4 \pi \times \text{area}) / \text{perimeter}^2]$ [13] (*figure 1*).

The average fruit weight (g) was obtained by collecting 20 randomly mature fruits from the four cardinal points in the canopy; then, these fruits were individually measured with a digital bascule. All data from all variables were registered in 2005.

The calculated correlations were done based on Pearson's correlation moment-product formula, with SAS V8's correlation procedure [14].

Screening of *Crataegus* spp. germplasm

Table I.

Collector's data for accessions of *Crataegus* from the germplasm bank of tejocote (*Crataegus* spp.) at the Universidad Autónoma Chapingo (Mexico).

Accession No.	Code	Place of collection	Latitude	Longitude	Altitude (m)	Taxonomic identification	Collector
			Decimal coordinates				
1	RNU02	Rancho Nuevo, Chiapas	16.67	-92.57	2400	<i>C. stipulosa</i>	Borys, 1982
2	RNU06	Rancho Nuevo, Chiapas	16.67	-92.57	2400	<i>C. stipulosa</i>	Borys, 1982
3	SCC02	San Cristóbal de las Casas, Chiapas	16.75	-92.67	2300	<i>C. stipulosa</i>	Borys, 1982
4	SCC01	San Cristóbal de las Casas, Chiapas	16.75	-92.67	2300	<i>C. stipulosa</i>	Borys, 1982
5	RNU05	Rancho Nuevo, Chiapas	16.67	-92.57	2400	<i>C. stipulosa</i>	Borys, 1982
6	RNU01	Rancho Nuevo, Chiapas	16.67	-92.57	2400	<i>C. stipulosa</i>	Borys, 1982
7	RRO02	Rancho Robelo, Chiapas	16.67	-92.45	2250	<i>C. stipulosa</i>	Borys, 1982
8	SCC05	San Cristóbal de las Casas, Chiapas	16.75	-92.67	2300	<i>C. stipulosa</i>	Borys, 1982
9	RNU04	Rancho Nuevo, Chiapas	16.67	-92.57	2400	<i>C. stipulosa</i>	Borys, 1982
10	SCC04	San Cristóbal de las Casas, Chiapas	16.75	-92.67	2300	<i>C. stipulosa</i>	Borys, 1982
12	RRO01	Rancho Robelo, Chiapas	16.67	-92.45	2250	<i>C. nelsoni</i>	Borys, 1982
13	RNU03	Rancho Nuevo, Chiapas	16.67	-92.57	2400	<i>C. nelsoni</i>	Borys, 1982
14	RRO06	Rancho Robelo, Chiapas	16.67	-92.45	2250	<i>C. nelsoni</i>	Borys, 1982
15	MIT02	Mitzitan, Chiapas	16.65	-92.55	2380	<i>C. nelsoni</i>	Borys and Nieto, 1985
16	MIT01	Mitzitan, Chiapas	16.65	-92.55	2380	<i>C. nelsoni</i>	Borys and Nieto, 1985
17	RNU07	Rancho Nuevo, Chiapas	16.67	-92.57	2400	<i>C. stipulosa</i>	Borys, 1982
18	RRO03	Rancho Robelo, Chiapas	16.67	-92.45	2250	<i>C. nelsoni</i>	Borys, 1982
19	MIT04	Mitzitan, Chiapas	16.65	-92.55	2380	<i>C. nelsoni</i>	Borys and Nieto, 1985
20	MIT05	Mitzitan, Chiapas	16.65	-92.55	2380	<i>C. nelsoni</i>	Borys and Nieto, 1985
21	RRO04	Rancho Robelo, Chiapas	16.67	-92.45	2250	<i>C. nelsoni</i>	Borys, 1982
22	MIT03	Mitzitan, Chiapas	16.65	-92.55	2380	<i>C. nelsoni</i>	Borys and Nieto, 1985
23	RNU08	Rancho Nuevo, Chiapas	16.67	-92.57	2400	<i>C. nelsoni</i>	Borys, 1982
24	CALP5	Calpan, Puebla	19.10	-98.47	2420	<i>C. mexicana</i>	Nieto and Borys, 1983
25	CALP6	Calpan, Puebla	19.10	-98.47	2420	<i>C. sp. *</i>	Nieto and Borys, 1983
26	CALP3	Calpan, Puebla	19.10	-98.47	2420	<i>C. gracilior</i>	Nieto and Borys, 1983
27	RRO05	Rancho Robelo, Chiapas	16.67	-92.45	2250	<i>C. nelsoni</i>	Borys, 1982
28	SPI01	San Pablo Ixayoc, Mexico	19.47	-98.78	2600	<i>C. mexicana</i>	Nieto and Borys, 1982
29	BAT01	Batan, Mexico	19.53	-98.83	2249	<i>C. gracilior</i>	Nieto, 1983
30	CALP4	Calpan, Puebla	19.10	-98.47	2420	<i>C. gracilior</i>	Nieto and Borys, 1983
31	CALP1	Calpan, Puebla	19.10	-98.47	2420	<i>C. gracilior</i>	Nieto and Borys, 1983
32	BAT02	Batan, Mexico	19.53	-98.83	2249	<i>C. gracilior</i>	Nieto, 1983
33	HUE01	Huejotzingo, Puebla	19.17	-98.4	2280	<i>C. mexicana</i>	Nieto and Borys, 1983
34	SNTP2	San Nicolás Tlaminas, Mexico	19.52	-98.75	2520	<i>C. gracilior</i>	Nieto and Borys, 1982
35	MIT07	Mitzitan, Chiapas	16.65	-92.55	2380	<i>C. stipulosa</i>	Borys and Nieto, 1985
36	SPI03	San Pablo Ixayoc, Mexico	19.47	-98.78	2600	<i>C. mexicana</i>	Nieto and Borys, 1982
37	SPI04	San Pablo Ixayoc, Mexico	19.47	-98.78	2600	<i>C. gracilior</i>	Nieto and Borys, 1982
38	SNTC1	San Nicolás Tlaminas, Mexico	19.52	-98.75	2520	<i>C. mexicana</i>	Nieto and Borys, 1982
39	SNTP1	San Nicolás Tlaminas, Mexico	19.52	-98.75	2520	<i>C. gracilior</i>	Nieto and Borys, 1982
40	SPI02	San Pablo Ixayoc, Mexico	19.47	-98.78	2600	<i>C. mexicana</i>	Nieto and Borys, 1982
41	SNTC2	San Nicolás Tlaminas, Mexico	19.52	-98.75	2520	<i>C. mexicana</i>	Nieto and Borys, 1982
42	SCC03	San Cristóbal de las Casas, Chiapas	16.75	-92.67	2300	<i>C. sp. *</i>	Borys, 1982
43	RRO07	Rancho Robelo, Chiapas	16.67	-92.45	2250	<i>C. sp. *</i>	Borys, 1982

Table I.
Continued.

Accession No.	Code	Place of collection	Latitude	Longitude	Altitude (m)	Taxonomic identification	Collector
			Decimal coordinates				
44	MIT06	Mitzitan, Chiapas	16.65	-92.55	2380	<i>C. gracilior</i>	Borys and Nieto, 1985
45	HUE02	Huejotzingo, Puebla	19.17	-98.40	2280	<i>C. gracilior</i>	Nieto and Borys, 1983
46	CALP2	Calpan, Puebla	19.10	-98.47	2420	<i>C. gracilior</i>	Nieto and Borys, 1983
47	HUE03	Huejotzingo, Puebla	19.17	-98.40	2280	<i>C. sp. *</i>	Nieto and Borys, 1983
48	HUE04	Huejotzingo, Puebla	19.17	-98.40	2280	<i>C. mexicana</i>	Nieto and Borys, 1983
49	SNTC4	San Nicolás Tlaminas, Mexico	19.52	-98.75	2520	<i>C. mexicana</i>	Nieto and Borys, 1982
50	SNTC3	San Nicolás Tlaminas, Mexico	19.52	-98.75	2520	<i>C. mexicana</i>	Nieto and Borys, 1982
51	MIT08	Mitzitan, Chiapas	16.65	-92.55	2380	<i>C. nelsoni</i>	Borys and Nieto, 1985
52	CAN01	Candelaria, Chiapas	16.70	-92.53	2320	<i>C. nelsoni</i>	Nieto and Barrientos, 1989
54	HUE05	Huejotzingo, Puebla	19.17	-98.40	2280	<i>C. mexicana</i>	Nieto and Borys, 1983
55	HUE06	Huejotzingo, Puebla	19.17	-98.40	2280	<i>C. gracilior</i>	Nieto and Borys, 1983
56	HUE07	Huejotzingo, Puebla	19.17	-98.40	2280	<i>C. gracilior</i>	Nieto and Borys, 1983
57	SCM01	Santa Catarina del Monte, Mexico	19.48	-98.77	2700	<i>C. gracilior</i>	Nieto and Borys, 1982
58	SCM02	Santa Catarina del Monte, Mexico	19.48	-98.77	2700	<i>C. gracilior</i>	Nieto and Borys, 1982
59	CAN02	Candelaria, Chiapas	16.70	-92.53	2320	<i>C. stipulosa</i>	Nieto and Barrientos, 1989
60	HUE08	Huejotzingo, Puebla	19.17	-98.40	2280	<i>C. gracilior</i>	Nieto and Borys, 1983
61	HUE09	Huejotzingo, Puebla	19.17	-98.40	2280	<i>C. gracilior</i>	Nieto and Borys, 1983
62	TEQ01	Tequexquahuac, Mexico	19.48	-98.82	2460	<i>C. gracilior</i>	Nieto and Borys, 1982
63	TEQ02	Tequexquahuac, Mexico	19.48	-98.82	2460	<i>C. gracilior</i>	Nieto and Borys, 1982
64	XAM01	Xamimilulco, Puebla	19.22	-98.38	2220	<i>C. gracilior</i>	Nieto and Barrientos, 1988
65	TEQ03	Tequexquahuac, Mexico	19.48	-98.82	2460	<i>C. gracilior</i>	Nieto and Borys, 1982
66	SCM03	Santa Catarina del Monte, Mexico	19.48	-98.77	2700	<i>C. gracilior</i>	Nieto and Borys, 1982
67	CAN03	Candelaria, Chiapas	16.70	-92.53	2320	<i>C. gracilior</i>	Nieto and Barrientos, 1989
68	CAN04	Candelaria, Chiapas	16.70	-92.53	2320	<i>C. nelsoni</i>	Nieto and Barrientos, 1989
69	CAN05	Candelaria, Chiapas	16.70	-92.53	2320	<i>C. nelsoni</i>	Nieto and Barrientos, 1989
70	CAN06	Candelaria, Chiapas	16.70	-92.53	2320	<i>C. nelsoni</i>	Nieto and Barrientos, 1989
71	SJY01	San José Yashitinin, Chiapas	16.65	-92.45	2350	<i>C. sp. *</i>	Nieto and Barrientos, 1989
72	SJY02	San José Yashitinin, Chiapas	16.65	-92.45	2350	<i>C. sp. *</i>	Nieto and Barrientos, 1989
73	SJY03	San José Yashitinin, Chiapas	16.65	-92.45	2350	<i>C. stipulosa</i>	Nieto and Barrientos, 1989
74	SJY04	San José Yashitinin, Chiapas	16.65	-92.45	2350	<i>C. nelsoni</i>	Nieto and Barrientos, 1989
75	SJY05	San José Yashitinin, Chiapas	16.65	-92.45	2350	<i>C. nelsoni</i>	Nieto and Barrientos, 1989
76	SJY06	San José Yashitinin, Chiapas	16.65	-92.45	2350	<i>C. nelsoni</i>	Nieto and Barrientos, 1989
77	SJY07	San José Yashitinin, Chiapas	16.65	-92.45	2350	<i>C. nelsoni</i>	Nieto and Barrientos, 1989
78	SJY08	San José Yashitinin, Chiapas	16.65	-92.45	2350	<i>C. sp. *</i>	Nieto and Barrientos, 1989
79	SJY09	San José Yashitinin, Chiapas	16.65	-92.45	2350	<i>C. sp. *</i>	Nieto and Barrientos, 1989
80	SCC06	San Cristóbal de las Casas, Chiapas	16.75	-92.67	2300	<i>C. nelsoni</i>	Nieto and Barrientos, 1989
81	SCC07	San Cristóbal de las Casas, Chiapas	16.75	-92.67	2300	<i>C. nelsoni</i>	Nieto and Barrientos, 1989
82	SCC08	San Cristóbal de las Casas, Chiapas	16.75	-92.67	2300	<i>C. nelsoni</i>	Nieto and Barrientos, 1989
83	SCC09	San Cristóbal de las Casas, Chiapas	16.75	-92.67	2300	<i>C. stipulosa</i>	Nieto and Barrientos, 1989
84	SCC10	San Cristóbal de las Casas, Chiapas	16.75	-92.67	2300	<i>C. stipulosa</i>	Nieto and Barrientos, 1989
85	SCC11	San Cristóbal de las Casas, Chiapas	16.75	-92.67	2300	<i>C. nelsoni</i>	Nieto and Barrientos, 1989
86	HUE10	Huejotzingo, Puebla	19.17	-98.40	2280	<i>C. mexicana</i>	Nieto and Barrientos, 1988

Table I.
Continued.

Accession No.	Code	Place of collection	Latitude	Longitude	Altitude (m)	Taxonomic identification	Collector
			Decimal coordinates				
88	SCC12	San Cristóbal de las Casas, Chiapas	16.75	- 92.67	2300	<i>C. stipulosa</i>	Nieto and Barrientos, 1989
89	SCC13	San Cristóbal de las Casas, Chiapas	16.75	- 92.67	2300	<i>C. stipulosa</i>	Nieto and Barrientos, 1989
92	XAM03	Xamimilulco, Puebla	19.22	- 98.38	2220	<i>C. gracilior</i>	Nieto and Barrientos, 1988
93	XAM04	Xamimilulco, Puebla	19.22	- 98.38	2220	<i>C. gracilior</i>	Nieto and Barrientos, 1988
94	ATX01	Atexcac, Puebla	19.13	- 98.50	2600	<i>C. gracilior</i>	Nieto and Barrientos, 1988
95	ATX02	Atexcac, Puebla	19.13	- 98.50	2600	<i>C. gracilior</i>	Nieto and Barrientos, 1988
96	ATX03	Atexcac, Puebla	19.13	- 98.50	2600	<i>C. gracilior</i>	Nieto and Barrientos, 1988
97	SCM04	Santa Catarina del Monte, Mexico	19.48	- 98.77	2700	<i>C. gracilior</i>	Nieto and Borys, 1982
99	SCC14	San Cristóbal de las Casas, Chiapas	16.75	- 92.67	2300	<i>C. nelsoni</i>	Nieto and Barrientos, 1989
100	HUE12	Huejotzingo, Puebla	19.17	- 98.40	2280	<i>C. mexicana</i>	Nieto 1989

* These accessions cannot be identified by Phipps [2] and Eggleston [1] taxonomic keys.

Table II.

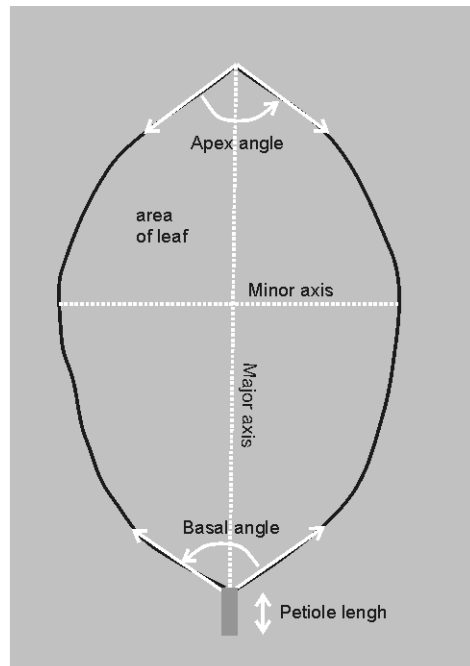
Young mature leaf traits studied from shoots of the middle part of tejocote (*Crataegus* spp.) tree (figure 1).

Character	Large vegetative shoots (> 7 cm)	Short vegetative shoots (< 7 cm)	Reproductive shoots
Apex angle from the leaf	Yes	Yes	Yes
Basal angle from the leaf	Yes	Yes	Yes
Area of leaf	Yes	Yes	Yes
Feret diameter	Yes	Yes	Yes
Elongation ratio	Yes	Yes	Yes
Compactness ratio	Yes	Yes	Yes
Roundness ratio	Yes	Yes	Yes
Major axis length	Yes	Yes	Yes
Minor axis length	Yes	Yes	Yes
Petiole length	Yes	Yes	Yes
Number of superficial teeth	No	No	Yes
Number of veins	No	Yes	Yes
Perimeter of the leaf	Yes	Yes	Yes
Distance between the closest stomata	No	No	Yes
Stomata frequency	No	No	Yes
Stomata length	No	No	Yes

The variables that showed a statistically significant correlation (positive or negative) with the average fruit weight, that were least modified by the environment, and that

allowed easy measurement, were identified. These variables are proposed as morphological markers for germplasm collection or early selection for fruit yield.

Figure 1.
Some leaf variables used for screening of tejocote (*Crataegus* spp.) germplasm.



3. Results

For the data obtained from all 94 accessions, the variables that showed a positive and statistically significant correlation with average fruit weight ($P \leq 0.01$) were (table III):

- area of one leaf from large vegetative shoots ($r = 0.70593^{**}$) and reproductive shoots ($r = 0.68736^{**}$),
- major axis length in leaves from reproductive shoots ($r = 0.70281^{**}$) and large vegetative shoots ($r = 0.69533^{**}$),
- Feret diameter of leaves from large vegetative shoots ($r = 0.65957^{**}$) and from reproductive shoots ($r = 0.65731^{**}$),
- number of veins in leaves from reproductive shoots ($r = 0.62302^{**}$).

On the other hand, the variables that were negatively correlated with average fruit weight, in ascendant order, were (table III):

- compactness ratio of leaves from reproductive, large and short vegetative shoots ($r = -0.39079^{**}$, $r = -0.38748^{**}$, and $r = -0.35827^{**}$, respectively),

- basal angle of leaves from short vegetative shoots ($r = -0.32656^{**}$),
- number of superficial teeth of leaves from reproductive shoots ($r = -0.34027^{**}$).

With respect to the correlations calculated for each species, some significant changes relative to the correlations for the general pool of *Crataegus* previously mentioned were observed (table III). These changes are more likely due to the intrinsic way of expression of the characters in each species (undoubtedly of genetic origin) which might be considerably variable among species, particularly fruit weight (table IV), and leaf and stomata variables. It is noteworthy, however, that *C. mexicana* DC. and *C. gracilior* Phipps show the largest fruit weight.

4. Discussion

The genus *Crataegus* was reported as one of the most difficult taxonomic complexes to be evaluated [10]. Because of this difficulty, to carry out breeding based on sexual crosses among selected genotypes is not a good strategy for tejocote. Furthermore, Núñez-Colín *et al.* [15] considered useful the development of selection indices to detect the appropriate individuals to be intercrossed to produce progenies with increased fruit size; but, because these species are polyploid and apomictic [5, 7, 9], Núñez-Colín *et al.* [15] concluded that making crosses among selected individuals is a waste of time and effort. Moreover, no studies were found relative to genetic parameters for this genus.

Dickinson and Phipps [16] demonstrated the leaf heteroblasty in *Crataegus crus-galli*, and, probably, Mexican species have this problem too, but there is no information about all of them. However, similarities between leaves of vegetative shoots and leaves of seedling plants may exist, and leaf traits of vegetative shoots can be used as morphological markers in seedling plants.

Nevertheless, taxonomical confusion exists even among taxonomists, mainly in distinguishing *C. mexicana* DC. from *C. gracilior* Phipps [correct name of *C. pubescens*

Table III.
Correlation coefficients of 36 leaf and 3 stomata variables with average fruit weight in tejocote (*Crataegus* spp.).

Character	Average fruit weight				
	All accessions	<i>C. gracilior</i>	<i>C. mexicana</i>	<i>C. nelsoni</i>	<i>C. stipulosa</i>
Area of leaf from large vegetative shoots	$r = 0.70593^{**}$	$r = 0.0477$	$r = 0.56335^{**}$	$r = 0.06157$	$r = 0.23663$
Major axis length in leaves from reproductive shoots	$r = 0.70281^{**}$	$r = 0.32033$	$r = 0.53154$	$r = -0.01542$	$r = 0.38075$
Major axis length in leaves from large vegetative shoots	$r = 0.69533^{**}$	$r = 0.10518$	$r = 0.63324^*$	$r = 0.07947$	$r = 0.28628$
Area of leaf from reproductive shoots	$r = 0.68736^{**}$	$r = 0.33054$	$r = 0.38755$	$r = -0.10835$	$r = 0.37375$
Feret diameter of leaves from large vegetative shoots	$r = 0.65957^{**}$	$r = 0.06644$	$r = 0.55559$	$r = 0.16843$	$r = 0.33511$
Feret diameter of leaves from reproductive shoots	$r = 0.65731^{**}$	$r = 0.30462$	$r = 0.39905$	$r = -0.00814$	$r = 0.43338$
Number of veins in leaves from reproductive shoots	$r = 0.62302^{**}$	$r = 0.45882^*$	$r = 0.5987^*$	$r = -0.17672$	$r = 0.5635^*$
Perimeter of leaf from reproductive shoots	$r = 0.61304^{**}$	$r = 0.28421$	$r = 0.35596$	$r = 0.07105$	$r = 0.3379$
Number of veins in leaves from short vegetative shoots	$r = 0.61034^{**}$	$r = 0.27312$	$r = 0.03501$	$r = 0.09181$	$r = 0.33191$
Perimeter of leaf from large vegetative shoots	$r = 0.57462^{**}$	$r = 0.04063$	$r = 0.61256^*$	$r = 0.27651$	$r = 0.22316$
Minor axis length in leaves from reproductive shoots	$r = 0.56435^{**}$	$r = 0.26765$	$r = 0.29444$	$r = 0.01312$	$r = 0.42398$
Major axis length in leaves from short vegetative shoots	$r = 0.56019^{**}$	$r = 0.04145$	$r = 0.07208$	$r = 0.02588$	$r = 0.3926$
Minor axis length in leaves from large vegetative shoots	$r = 0.55662^{**}$	$r = 0.02257$	$r = 0.51545$	$r = 0.19337$	$r = 0.28745$
Area of leaf from short vegetative shoots	$r = 0.53434^{**}$	$r = 0.01367$	$r = 0.02938$	$r = 0.02234$	$r = 0.31176$
Elongation ratio of leaves from reproductive shoots	$r = 0.49247^{**}$	$r = 0.32848$	$r = 0.69794^{**}$	$r = 0.01059$	$r = -0.18879$
Feret diameter of leaves from short vegetative shoots	$r = 0.48497^{**}$	$r = 0.01019$	$r = 0.07692$	$r = 0.10147$	$r = 0.39407$
Elongation ratio of leaves from large vegetative shoots	$r = 0.47262^{**}$	$r = 0.34605$	$r = 0.66057^*$	$r = -0.05752$	$r = -0.07595$
Petiole length in leaves from large vegetative shoots	$r = 0.39881^{**}$	$r = 0.00212$	$r = 0.64557^*$	$r = 0.015$	$r = -0.01847$
Elongation ratio of leaves from short vegetative shoots	$r = 0.39358^{**}$	$r = 0.3915^*$	$r = 0.14342$	$r = -0.06596$	$r = 0.05833$
Roundness ratio of leaves from short vegetative shoots	$r = 0.38889^{**}$	$r = 0.03507$	$r = 0.64637^*$	$r = -0.44137^*$	$r = 0.09891$
Perimeter of leaf from short vegetative shoots	$r = 0.3801^{**}$	$r = -0.00097$	$r = -0.01572$	$r = 0.21529$	$r = 0.28273$
Minor axis length in leaves from short vegetative shoots	$r = 0.3561^{**}$	$r = -0.03791$	$r = 0.05933$	$r = 0.14501$	$r = 0.34863$
Petiole length in leaves from reproductive shoots	$r = 0.34555^{**}$	$r = 0.30822$	$r = 0.60306^*$	$r = 0.10417$	$r = -0.04711$
Roundness ratio of leaves from large vegetative shoots	$r = 0.31585^{**}$	$r = 0.01087$	$r = 0.11775$	$r = -0.41539^*$	$r = 0.15102$
Petiole length in leaves from short vegetative shoots	$r = 0.199$	$r = -0.08403$	$r = 0.20471$	$r = 0.04655$	$r = 0.00471$
Stomata frequency in leaves from reproductive shoots	$r = 0.19197$	$r = -0.0376$	$r = 0.18821$	$r = -0.50228^{**}$	$r = 0.09734$
Roundness ratio of leaves from reproductive shoots	$r = 0.14012$	$r = -0.12581$	$r = 0.35371$	$r = -0.45984^*$	$r = -0.00243$
Basal angle in leaves from reproductive shoots	$r = 0.03651$	$r = 0.3468$	$r = -0.56661^*$	$r = -0.23384$	$r = 0.24305$
Apex angle in leaves from short vegetative shoots	$r = -0.03105$	$r = -0.43739^*$	$r = 0.0862$	$r = 0.40406^*$	$r = 0.03441$
Distance between closer stomata in leaves from reproductive shoots	$r = -0.1102$	$r = 0.20409$	$r = -0.05617$	$r = 0.51243^{**}$	$r = 0.01772$
Apex angle in leaves from reproductive shoots	$r = -0.1888$	$r = -0.43872^*$	$r = -0.43622$	$r = 0.14367$	$r = 0.32586$
Basal angle in leaves from large vegetative shoots	$r = -0.20005$	$r = 0.18373$	$r = -0.49389$	$r = 0.17371$	$r = 0.27522$
Apex angle in leaves from large vegetative shoots	$r = -0.22153^*$	$r = -0.50376^{**}$	$r = -0.48521$	$r = 0.42634^*$	$r = 0.20074$
Stomata length in leaves from reproductive shoots	$r = -0.25079^*$	$r = -0.31559$	$r = -0.40959$	$r = 0.38077$	$r = 0.25551$
Basal angle in leaves from short vegetative shoots	$r = -0.32656^{**}$	$r = 0.09358$	$r = -0.41734$	$r = 0.09902$	$r = 0.11209$
Number of superficial teeth in leaves from reproductive shoots	$r = -0.34027^{**}$	$r = -0.04789$	$r = -0.44594$	$r = -0.3658$	$r = 0.01997$
Compactness ratio of leaves from short vegetative shoots	$r = -0.35827^{**}$	$r = -0.33501$	$r = -0.11902$	$r = 0.09881$	$r = 0.01382$
Compactness ratio of leaves from large vegetative shoots	$r = -0.38748^{**}$	$r = -0.34046$	$r = -0.65982^*$	$r = 0.13663$	$r = 0.19825$
Compactness ratio of leaves from reproductive shoots	$r = -0.39079^{**}$	$r = -0.26965$	$r = -0.6728^*$	$r = -0.08115$	$r = 0.31213$

*, ** Statistically significant with probability of $P = 0.05$ and $P = 0.01$, respectively.

(H. B. K.) Steud. and this species belongs to the same series as *C. crus-galli*, despite the fact that Eggleston [1] has described them as different species since the beginning of the twentieth century. Both species

grow side by side (sympatrically) in the few commercial orchards of tejocote. In this case, to search for propagation material of tejocote, the researcher could select a genotype based on the morphometric traits

Table IV.

Mean, maximum, minimum, and standard deviation of fruit weight (g) in each of the four species of *Crataegus* studied.

Taxonomic specie	Mean	Maximum	Minimum	Standard deviation
<i>C. gracilior</i>	5.49	10.34	2.72	2.02
<i>C. mexicana</i>	11.51	19.36	7.36	3.08
<i>C. nelsoni</i>	2.67	4.33	0.81	1.01
<i>C. stipulosa</i>	3.39	5.57	1.40	1.06

correlated with fruit weight, even without identifying the taxonomic species, especially if the researcher does not have experience in *Crataegus* taxonomy. Moreover, to breed *Crataegus* species for fruit quality, the starting point should be the superior germplasm that is available or collectable [17], mainly because *Crataegus mexicana* DC., native to Mexico, is one of the oldest species of the genus, embraces high variability [18] in fruit characters, and possesses the biggest *Crataegus* fruit reported [10].

When collecting superior germplasm of tejocote, two cases are possible:

- First, when the taxonomic identity is available, the best option is to select *C. mexicana* because this species has the best fruit characteristics such as size, weight and shape (table IV) [10, 19]. Then, in the retained *C. mexicana* individuals, the traits to be considered for selection are large and elongated leaves with at least 15 veins, because these variables are the most highly correlated with fruit weight (table III), and, thereby, they allow indirect selection of superior germplasm, even at the juvenile stage.
- The second scenario is when the taxonomic identity is unknown. *Crataegus mexicana* is easily confused with *C. gracilior* because they only differ in a few characters such as pubescence in leaves (*C. mexicana* is more pubescent), inflorescence pubescence (*C. mexicana* is densely pubescent), calyx remnants in the fruit (only present in *C. mexicana*) and number of endocarps (*C. gracilior* shows 2 or 3 of them, while *C. mexicana* shows 4 or 5). These two species are more

easily distinguishable from other species because they are very wild and their fruits are very small (table IV) [1, 2, 10, 19].

The most important trait correlated with fruit weight in the two most important species is the number of veins (at least 15 veins are desirable). In addition, as the first scenario, this variable is measurable even at the juvenile stage, to select indirectly superior germplasm for fruit weight. Obviously, early selection of genotypes would save time.

5. Conclusions

Selection for fruit weight in species of the *Crataegus* genus where the taxonomic identity is not clearly defined should be based on morphological markers, particularly on the presence of leaves with at least 15 veins. Further, whenever the species are distinguishable, *C. mexicana* germplasm should be selected, and then plants whose leaves have at least 15 veins, are large (at least 7 cm), and are elongated (an elongation ratio of 2 or greater) have to be identified.

Acknowledgements

The study was supported in part by the scholarship 169581 from the Mexican National Council for Science and Technology (CONACYT). The authors specially acknowledge the anonymous referees for their comments on this paper.

References

- [1] Eggleston W.W., The *Crataegi* of Mexico and Central America, Bull. Torrey Bot. Club 36 (1909) 501–514.
- [2] Phipps J.B., Monograph of Northern Mexican *Crataegus* (Rosaceae subfam. Maloideae), SIDA Bot. Misc. 15 (1997) 1–94.
- [3] Núñez-Colín C.A., Pérez-Ortega S.A., Segura S., Nieto-Ángel R., Barrientos-Priego A.F., Variabilidad morfológica de tejocote (*Crataegus* spp.) en México, Proc. Interam. Soc. Trop. Hortic. 48 (2004) 144–148.
- [4] Pérez-Ortega S.A., Núñez-Colín C.A., Segura S., Nieto-Ángel R., Barrientos-Priego A.F., Los recursos genéticos de *Crataegus* (Rosaceae) en México: Variación eco-climática, Proc. Interam. Soc. Trop. Hortic. 48 (2004) 149–151.
- [5] Talent N., Dickinson T.A., Polyploidy in *Crataegus* and *Mespilus* (Rosaceae, Maloideae): evolutionary inference from flow cytometry of nuclear DNA amounts, Can. J. Bot. 83 (2005) 1268–1304.
- [6] Grant V., Especiación vegetal, Editor. Limusa, Mex. city, Mex., 1989.
- [7] Talent N., Dickinson T.A., Apomixis and hybridization in Rosaceae subtribe Pyrineae Dumort.: a new tool promises new insights, In: Hörandl E., Grossniklaus U., Van Dijk P.J., Sharbel T. (Eds.), Apomixis: evolution, mechanisms and perspectives, Int. Assoc. Plant Taxon. and Koeltz Sci. Books, Vienna, Austria, 2007, pp. 301–316.
- [8] Muniyamma M., Phipps J.B., Cytological proof of apomixis in *Crataegus* (Rosaceae), Am. J. Bot. 66 (2) (1979) 149–155.
- [9] Talent N., Dickinson T.A., Endosperm formation in aposporous *Crataegus* (Rosaceae, Spiraeoideae, tribe Pyreae): parallels to Ranunculaceae and Poaceae, New Phytol. 173 (2007) 231–249.
- [10] Phipps J.B., O’Kennon R.J., Lance R.W., Hawthorns and medlars, Timber Press, Portland, USA, 2003.
- [11] El-Gazaar A., The taxonomic significance of leaf morphology in *Crataegus* (Rosaceae), Bot. Jahrb. Syst. 101 (1980) 457–469.
- [12] Way R.D., Sanford J.C., Lakso A.N., Fructificación y productividad, in: Moore J.N., Janick J. (Eds.), Métodos genotécnicos en frutales, AGT Ed., Mexico city, Mexico, 1988, pp. 477–495.
- [13] Wilcox D., Dove B., McDavid D., Greer D., Image tool Version 3, Users guide, Univ. Tex. Health Sci. Cent., San Antonio, USA, 1995.
- [14] Anon., SAS/STAT software: changes and enhancements through release 8, SAS Inst., Cary, USA, 1999.
- [15] Núñez-Colín C.A., Nieto-Ángel R., Barrientos-Priego A.F., Índices de selección para aumentar tamaño de fruto en tejocote (*Crataegus* spp.), Proc. Interam. Soc. Trop. Hortic. 49 (2005) 146–148.
- [16] Dickinson T.A., Phipps J.B., Studies in *Crataegus* (Rosaceae, Maloideae). IX. Short shoot leaf heteroblasty in *Crataegus crus-galli sensu lato*, Can. J. Bot. 62 (1984) 1775–1780.
- [17] Zagaja S.W., Exploración de recursos genéticos, in: Moore J.N., Janick J. (Eds.), Métodos genotécnicos en frutales, AGT Ed., Mex. city, Mex., 1988, pp. 3–12.
- [18] Phipps J.B., Problems of hybridity in the clastics of *Crataegus* (Rosaceae), in: Grant W.F. (Ed.), Plant Biosystematics, Acad. Press Can., Tor., Can., 1984, pp. 417–438.
- [19] Büttner R., Rosaceae, in: P. Hanelt and Inst. Plant Genet. Crop Plant Res. (Eds.), Mansfeld’s Encycl. Agric. Hortic. Crops: except ornam., Springer, Berlin-Heidelberg-New York, 2001, pp. 417–532.

Identificación de características morfométricas para la selección de germoplasma de tejocote (*Crataegus* spp.) con un mejor potencial de rendimiento.

Resumen — Introducción. El tejocote (*Crataegus* spp.) es un frutal que algunas culturas mesoamericanas cultivaban desde épocas prehispánicas en México; sin embargo, esta especie carece de mejoramiento genético formal. Se desconoce si existen marcadores morfológicos asociados al tamaño de fruto que pudieran ser utilizados para selección indirecta; que pueden ser importantes por ser una especie de ciclo largo. Esta investigación tuvo como objetivo determinar el valor de las correlaciones fenotípicas entre caracteres de hoja y estomas con el peso de fruto, como marcadores morfológicos para la selección indirecta de germoplasma superior en rendimiento de fruto. **Material y métodos.** Se estimó el coeficiente de correlación de Pearson de 36 variables de hoja y tres de estomas con el peso de fruto en 94 genotipos de cuatro especies de *Crataegus*. **Resultados.** Como se esperaba, las correlaciones difieren en las especies y en el conjunto de especies. Sin embargo, el número de venas de hojas de brotes reproductivos se correlacionó positiva y significativamente ($P \leq 0,05$) con el peso de fruto en las dos especies hortícolas más importantes. Por otra parte, *C. mexicana* es la mejor opción para seleccionar germoplasma superior para peso de fruto y las variables más correlacionadas con esta variable son la longitud del eje mayor de hojas de brotes vegetativos largos, el índice de elongación y el número de venas en hojas de brotes reproductivos. **Discusión.** Estas variables podrían ser buenos marcadores morfológicos de selección temprana, puesto que se pueden seleccionar plantas sin producción de frutos, que pueden llegar a tener una producción satisfactoria.

México / *Crataegus* spp. / fitomejoramiento / elección de especies / criterios de selección / frutas / rendimiento

