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Coffee (*Coffea arabica* L.) harvesting time and its influence on the seed quality of the Costa Rica 95 and Garnica varieties

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Contacto principal
Jorge Cadena Iñiguez
Guerrero 9, esquina avenida Hidalgo,
C.P. 56220, San Luis Huexotla, Texcoco,
Estado de México.
agroproductividadeditor@gmail.com

Contacto de soporte
Soporte
5959284703
agroproductividadsoporte@gmail.com

Directrices para Autores/as

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Heterosis and agronomic performance of raspberry families

Hernández-Bautista, Aurelio[1]; Lobato-Ortiz, Ricardo[1*]; Mejía-Contreras, José Apolinar[1]; García-Zavala, J. Jesús[1]; Chávez-Servia, José Luis[2]; Rocandio-Rodríguez, Mario[3]; Hernández-Bautista, Ana Lidia[4]; Castañeda-Villa, Alba Melissa[1]; Rodríguez-Bautista, Geremias[5]

[1] Colegio de Postgraduados Campus Montecillo. Carretera México-Texcoco km 36.5, Montecillo, Texcoco, Estado de México, México. C. P. 56230.

[2] Instituto Politécnico Nacional, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional-Unidad Oaxaca. Hornos No. 1003, Santa Cruz Xoxocotlán, Oaxaca, México. C. P. 71230.

[3] Universidad Autónoma de Tamaulipas. Unidad Académica Multidisciplinaria Agronomía y Ciencias. Centro Universitario Adolfo López Mateos. Cd. Victoria, Tamaulipas, México. C. P. 87149.

[4] Universidad del Golfo de México, Campus Norte. Adolfo Ruiz Cortines S/N, Martínez de la Torre, Veracruz, México. C. P. 93607.

[5] Universidad de Guadalajara, Centro Universitario de la Costa Sur. Av. Independencia Nacional #151, Autlán, Jalisco, México. C. P. 48900.

* Correspondence: rlobato@colpos.mx

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ABSTRACT

Objective: To study the heterosis and agronomic performance of raspberry (*Rubus idaeus*) F_1 families derived from open-pollinated parents, and to investigate the heterotic relationships between yield and its components.

Design/methodology/approach: A total of thirty-five genotypes, including eight open-pollinated raspberry cultivars, their 28 F_1 families and one check, were evaluated for vegetative and fruit traits. The trial was carried out under a randomized block design and under open field conditions.

Results: Mid-parent heterosis (MH) ranged from −94.83 to 311.67 %, whereas the better parent heterosis (BPH) values varied from −94.26 to 235.00%. We observed that the heterosis values for yield had a strong and positive correlation (r=0.89) with the heterosis values for number of fruits per plant.

Limitations on study/implications: Heterosis and performance of F_1 families in raspberry would depend on the pedigree of parents as their relativeness is a key factor to exploit the heterosis in plants.

Findings/conclusions: High values of heterosis were found in some raspberry crosses. Progeny derived from parents MU1 and TD865 showed considerable mid-parent heterosis (MH) and good performance for fruit size-related traits, soluble solids content and yield, evidencing that both parents may be utilized as donor parents in raspberry breeding program.

Keywords: Raspberry breeding, *Rubus idaeus*, heterosis, hybrid performance.

INTRODUCTION

In Mexico, raspberry (*Rubus idaeus* L.) has growth in total annual production, harvested area, and yield per hectare particularly in the states of Michoacán and Jalisco (SIAP, 2019). In the past, the 'Autumn bliss' variety was the most widely planted in Mexico.

Image by Vip_PinDos at Pixabay

Recently, the raspberry industry in Mexico have invested in the developing new varieties with better fruit quality (flavor, firmness, and color) and yield (Hernández-Bautista *et al.*, 2019). The development of new raspberry varieties is carried out employing traditional breeding schemes; 1) parents heterozygous are crossed manually, 2) individual selection is worked in the new F_1 families, 3) the best plants are propagated clonally by roots or *in vitro* techniques for a second evaluation, and 4) the best selections observed in the second evaluation are evaluated in a large trial named semi-commercial evaluation (Hernández-Bautista *et al.*, 2019).

Heterosis or hybrid vigor is defined as the phenomenon where the progeny derived from the cross of two parents shows superior performance than their parents (Acquaah 2007). Three hypotheses have been proposed to explain the phenomenon of heterosis. The dominance hypothesis, which is the hypothesis accepted most widely, explains that recessive genes of each inbred parent are masked by the dominant genes when these are inherited to F_1 progeny (Davenport, 1908). The second hypothesis, proposed by East (1980), is known as true overdominance and establishes that the heterozygote genotype is superior to its both homozygous parents due to the overexpression of the genes. Epistasis is considerate the third genetic model to explain heterosis. Epistasis particularly that involves dominance effects (dominance×dominance) has been reported as main factor conferring heterosis (Yu *et al.*, 1997).

Previous studies suggest that heterosis is positively related to the genetic distance observed between the two parents (Cox and Murphy, 1990). Therefore, it is common in the breeding programs to maintain two or more heterotic groups. In contrast, studies in cassava (Ceballos *et al.*, 2016), and pepper (Geleta *et al.*, 2004) reported a poor relationship between genetic distance and heterosis, suggesting heterosis is a complex phenomenal which is affected by genetic and environmental factors. The objectives of the paper were: 1) to study the degree of heterosis and agronomic performance of raspberry hybrids growth under open field conditions, and 2) to study the heterotic relationships among yield and its components.

MATERIALS AND METHODS

The plant material for this study consisted of eight open-pollinated raspberry parents, their F_1 families and one check. A total of 28 F_1 families were obtained under a diallel mating design without reciprocal crosses. The parental genotypes were CP65, CP47, TD865, MRSL, MU1, JG, JJ24 and CP57, and the check was one commercial variety named 'Autumn Bliss'. The trial was performed under open-field conditions and conducted from September 2015 to May 2016 in Ziracuaretiro, Michoacán, Mexico. Parents, F_1 hybrid families and one check were transplanted under a randomized complete blocks design with four replicates (26 plants per plot). The agronomic management for fertilization was worked following the recommendations for commercial production.

A total of nine traits quantitative traits were scored. Number of canes per plant and plant height were evaluated in each plant at 50% blooming stage. Number of berries per plant, berry weight (g), berry length (mm), berry width (mm) and total soluble solids (°Bx), were obtained from harvests that were worked two times weekly for two months.

Number of drupelets per berry was measured by counting the drupelets from 10 fruits with exportation quality. Finally, yield per plant was estimated as total weight obtained of all harvesters worked throughout the season.

Data were analyzed using the mean values of each genotype in each replication. Analysis of variance and Tukey's test were performed ($P \leq 0.05$). For each trait, components of phenotypic variance were estimated from analysis of variance using restricted maximum likelihood methods. Experimental data from field trial was analyzed using the following mixed linear model:

$$Y_{ij} = \mu + h_i + b_j + e_{ij}$$

where Y_{ij}, is the observed performance of the ith hybrid in the block j, μ is the overall mean, h_i is the random effect of hybrid i, b_j is the fixed effect of the block j and e_{ij} is the random residual term. The computation was performed using the PROC MIXED procedure in SAS Program version 9.3 (SAS Institute, 2012). The percentage heterosis based on mid-parent (MH) and better parent (BPH) were calculated using the following formulas:

$$Mid - parent\ heterosis = \frac{\left(F_1 - \left(\frac{P_1 + P_2}{2}\right)\right)}{\frac{P_1 + P_2}{2}} \times 100$$

$$Better - parent\ heterosis = \frac{\left(F_1 - (HP)\right)}{HP} \times 100$$

where: F_1=mean of F_1 family, P_1=mean of female parent, P_2=mean of male parent and HP=is the better parent value.

Finally, to know how the values of heterosis for yield are affected by the yield-components heterotic values, the Pearson's correlation coefficients were estimated using the mid-parent (MH) and better-parent heterosis (BPH) values. This analysis as performed employing PROC CORR in SAS program version 9.3 (SAS Institute, 2012).

RESULTS AND DISCUSSION

Analysis of variance detected significant differences for the factor genotypes on all studied traits (Table 1), demonstrating the presence of sufficient genetic variability among parents and families. These results were supported by estimated genetic variances. Yield per plant exhibited the highest proportion of genetic variance, whereas berry size-related traits (berry length and width) had the lowest levels of genetic variance. Number of berries per plant and plant height had a relatively high proportion of variance evidencing the wide gene pool presents in the population. Berry weight, number of canes per plant and soluble solids content had variance values <10, and number of drupelets >480. Similar results in phenotypic variation were reported by Fotirić-Akšić *et al.* (2011) and Stephens *et al.* (2012), who found a low phenotypic variance for berry weight, berry length, solid soluble content, berry width, and high variance for height plant and number of drupelets.

Some genotypes exhibited better traits than those observed on 'Autumn Bliss' under open field conditions (Table 2). For berry weight, MRSL exhibited with the highest value for this trait (13.25 g), followed by TD865×MRSL and TD865. In the case of number of berries per plant, the genotypes ranged from 10 fruits to 235 fruits. Sixteen F_1 families produced a higher number of berries compared with the check, and the family TD865×MU1 was the best. For yield, the highest values in the families were found on TD865×MRSL and MRSL×MU1, which exhibited values of 740.55 and 721.45 g, respectively, followed by CP47×CP57 and CP47×MU1.

Concerning to number of canes, the family MU-1×CP57 reflected the highest value (14 canes) for this characteristic whereas lowest value was obtained for the parental CP57. The highest values for plant height were noticed in plants of the genotypes CP47, CP65×JJ24, CP47×JG and CP47×CP57. For fruit-size related traits such as berry length, berry diameter and number of drupelets per berry, the best hybrid families that reflected the highest values were determined for the hybrid combinations TD865×MRSL for berry length; MRSL×JG for berry diameter, and MRSL×MU1 for number of drupelets per fruit. Finally, for soluble solids content, more than 50% of the genotypes exhibited higher values than the check, where crosses involving TD865 tended to show high soluble solids content followed by the TD865×JJ24.

The heterosis percentages values relative to mid-parent (MH) and better parent (BPH) are presented in Table 3. Across the entire experiment, about 40% of the crosses exhibited MH and a lower percentage of families showed a positive BPH for every trait. In total yield per plant, the MH ranged from −91.83 to 122.27%, whereas the BPH from −94.26 to

Table 1. Mean squares of analysis of variance and genetic components for yield and eight yield-related characteristics of the 37 evaluated genotypes of raspberry.

Trait	Mean Squares			C. V.	Variance component		
	Genotype	Replication	Error		σ^2_G †	σ^2_{REP}	σ^2_E
Yield per plant (g)	163435.93 **	3982.40	4942.64	17.68	39623.30	−25.95	4942.60
Number of berries per plant	11881.75 ***	387.56	456.57	20.09	2856.30	−1.87	456.57
Berry weight (g)	11.32 ***	0.27	0.21	10.24	2.78	0.00	0.21
Berry length (mm)	0.61 **	0.02	0.01	4.61	0.15	0.00	0.01
Berry width (mm)	0.60 ***	0.01	0.01	4.73	0.15	0.00	0.01
Number of drupelets per berry	1987.04 ***	69.67	41.81	7.44	486.31	0.75	41.81
Number of canes per plant	38.34 ***	1.21	0.99	14.29	9.34	0.01	0.99
Plant height (cm)	7407.27 ***	6.29	408.93	11.04	1749.60	−10.88	408.93
Total soluble solids (°Bx)	6.56 ***	1.16	0.42	6.06	1.53	0.02	0.42
d.f.	36	3	108				

†$\sigma\sigma^2_G$ =Genotypic variance; σ^2_{REP} =Blocks variance; σ^2_E =Environmental variance; f.=Degrees of freedom; **, *** indicate significant difference at P<0.01 and <0.001, respectively.

Table 2. Mean performance of 8 parents, 28 F_1 families and the check 'Autumn Bliss' for nine characters of raspberry.

Genotype	Yield per plant (g)	Number of berries per plant	Berry weight (g)	Berry length (mm)	Berry width (mm)	Number of drupelets per berry	Number of canes per plant	Plant height (cm)	Total soluble solids (Bx°)
CP65	248.98 j-p	55.45 l-p	3.98 e-i	2.01 g-j	2.15 d-h	69.75 l-m	8.40 d-h	241.25 a-e	11.13 b-h
CP47	472.86 d-i	126.21 c-j	4.55 c-h	2.49 c	2.51 c	98.25 c-h	8.70 d-g	*282.50* a	12.40 a-c
TD865	451.39 e-i	81.67 i-n	5.48 bc	2.46 cd	2.27 cd	*127.50* a	3.00 m-o	241.50 a-e	*13.70* a
MRSL	*904.17*[†] a	124.25 c-k	*13.25* a	*3.16* a	2.82 b	112.75 a-d	2.00 no	174.75 f-l	9.27 i-l
MU1	560.23 b-f	103.44 e-l	4.89 c-g	2.22 c-g	2.13 d-h	104.50 b-f	7.75 e-i	183.50 f-l	12.45 a-c
JG	209.54 l-q	59.50 l-p	3.80 f-k	2.01 g-j	2.15 d-h	69.75 l-n	4.00 k-o	241.25 a-e	11.28 b-f
JJ24	210.67 l-q	58.35 l-p	3.66 g-k	2.12 e-i	1.87 hi	92.25 e-j	8.25 d-h	166.25 f-m	10.49 d-j
CP57	518.70 d-h	174.90 bc	3.28 h-k	2.02 g-j	2.07 d-i	104.50 b-f	1.32 o	112.71 mn	7.59 l
CP65×CP47	405.65 f-l	111.60 e-l	3.91 f-j	2.10 f-i	2.17 d-g	78.56 i-m	9.84 b-e	144.90 i-n	11.33 b-f
CP65×TD865	428.80 f-k	136.45 c-h	3.91 f-j	2.10 f-i	2.13 d-h	82.37 g-l	7.40 e-j	200.85 c-i	10.58 d-j
CP65×MRSL	666.26 b-d	168.10 cd	4.46 c-h	2.85 b	3.14 a	111.79 a-d	10.60 b-c	152.33 g-n	10.55 d-j
CP65×MU1	246.55 j-p	70.20 j-o	3.88 f-j	2.07 g-i	2.15 d-h	88.39 f-k	5.33 i-m	134.56 k-n	11.39 b-f
CP65×JG	432.67 f-j	97.50 f-l	3.86 f-j	1.77 jk	1.90 f-i	64.04 m-o	3.00 m-o	182.40 f-l	10.13 e-j
CP65×JJ24	454.54 e-i	127.21 c-j	3.65 g-k	1.85 i-k	1.79 ij	71.66 k-n	4.00 k-o	265.15 ab	10.65 c-j
CP65×CP57	73.51 p-q	29.68 n-p	2.66 jk	1.72 kl	1.89 f-i	34.36 q	7.75 e-i	174.75 f-l	9.38 h-l
CP47×TD865	335.10 h-n	90.30 g-m	4.60 c-g	2.03 g-j	2.03 d-i	74.35 j-n	4.00 k-n	171.79 f-l	12.05 a-d
CP47×MRSL	279.70 i-n	64.85 k-p	5.23 c-e	2.04 g-j	1.97 e-i	75.46 j-n	11.75 a-n	142.29 j-n	9.90 f-j
CP47×MU1	517.10 d-h	224.10 ab	4.46 c-h	2.29 c-g	2.16 d-h	75.74 j-n	7.00 f-j	189.25 e-k	11.51 b-f
CP47×JG	113.00 o-q	30.75 m-p	3.69 g-k	2.18 d-h	2.26 cd	82.15 g-m	5.89 h-l	249.70 a-c	11.35 b-f
CP47×JJ24	471.60 d-i	138.10 c-h	4.14 d-i	2.37 c-f	2.22 c-e	81.06 h-m	8.00 d-i	145.85 i-n	10.99 b-i
CP47×CP57	535.10 c-g	168.85 b-d	3.79 f-k	2.28 c-g	2.10 d-h	96.27 d-i	11.60 a-c	245.95 a-d	11.64 b-e
TD865×MRSL	346.95 g-n	76.05 i-o	6.55 b	3.22 a	2.82 b	107.79 b-e	4.75 j-n	130.02 l-n	11.35 b-f
TD865×MU1	740.55 ab	*235.50* a	4.47 c-h	2.39 c-e	2.18 d-g	80.81 h-m	9.20 c-g	205.45 c-h	11.25 b-g
TD865×JG	352.45 g-n	132.00 c-i	3.67 g-k	2.09 f-i	2.01 d-i	82.28 g-l	9.65 b-f	172.75 f-l	10.18 e-j
TD865×JJ24	185.56 m-q	52.50 l-p	3.65 g-k	2.29 c-g	2.06 d-i	73.03 k-n	3.88 l-o	188.79 e-k	12.60 ab
TD865×CP57	235.63 k-p	62.25 l-p	3.75 f-k	2.24 c-g	2.18 d-f	115.25 a-c	2.25 no	189.44 d-k	10.15 e-j
MRSL×MU1	721.45 a-c	170.65 b-d	5.34 b-d	2.95 ab	3.12 a	126.51 a	10.00 b-e	150.03 h-n	10.50 d-j
MRSL×JG	631.10 b-e	154.40 c-f	4.68 c-g	3.03 ab	*3.40* a	121.90 ab	12.35 ab	160.50 g-n	9.44 g-k
MRSL×JJ24	489.30 d-h	158.05 c-e	4.59 c-g	2.12 e-i	1.95 e-i	82.11 g-m	7.40 e-j	160.80g-n	11.81 b-e
MRSL×CP57	366.65 f-m	105.70 e-l	4.22 c-i	1.45 l	1.56 j	48.00 o-q	6.70 g-k	136.40 k-n	8.85 j-l
MU1×JG	100.26 o-q	19.91 p-o	5.35 b-d	1.70 kl	1.89 g-i	44.23 pq	5.74 h-m	206.88 c-g	11.33 b-f
MU1×JJ24	639.75 b-e	142.75 c-g	4.36 c-i	2.22 c-g	2.23 c-e	58.15 n-p	8.08 d-i	197.50 c-j	11.13 b-h
MU1×CP57	162.88 n-q	74.13 i-o	2.55 k	2.14 e-h	2.18 d-g	107.77 b-e	*14.00* a	134.32 k-n	10.45 e-j
JG×JJ24	467.00 e-i	144.65 c-g	4.29 c-i	2.12 e-i	2.09 d-h	81.13 h-m	7.25 e-i	185.50 e-l	11.25 b-g
JG×CP57	29.75 q	10.05 p	3.10 i-k	1.90 h-k	2.00 d-i	85.47 g-l	7.85 d-i	219.00 b-f	9.45 g-k
JJ24×CP57	252.47 j-p	62.65 l-p	5.02 c-f	2.15 e-h	2.03 d-i	99.84 c-g	5.34 i-m	108.71 n	9.23 i-l
'Autumn Bliss'	458.59 e-i	92.50 g-l	4.68 c-g	2.22 c-g	1.93 f-i	104.50 b-f	4.00 k-o	190.77 d-k	7.80 kl
LSD	196.96	59.86	1.28	0.29	0.29	18.12	2.79	56.65	1.82
Mean	397.74	106.36	4.47	2.23	2.20	86.87	6.97	183.25	10.72

Genotypes sharing same letter are equal according to Tukey's test ($P<0.05$); †=Number in italics indicates the highest value for each case.

Table 3. Percentages of mid-parent (MH) and better parent (BPH) heterosis exhibited by F_1 hybrids for yield and eight yield-components.

Genotype	Yield per plant (g)		Number of berries per plant		Berry weight (g)		Berry length (mm)		Berry width (mm)		Number of drupelets per berry		Number of canes per plant		Plant height (cm)		Total soluble solids (°Bx)	
	MH	BPH	MH	BPH	MH	BPH	MH	BPH	MH	BPH	MH	BPH	MH	BPH	MH	BPH	MH	BPH
CP65×CP47	12.39	−14.21	22.87	−11.58	−8.27	−14.06	−6.94	−15.91	−6.62	−13.35	−6.48	−20.05	15.06	13.07	−44.67	−48.71	−3.68	−8.63
CP65×TD865	22.45	−5.00	99.02	67.07	−17.30	−28.63	−6.03	−14.66	−3.57	−6.22	−16.49	−35.40	29.82	−11.90	−16.79	−16.83	−14.81	−22.81
CP65×MRSL	15.56	−26.31	87.09	35.29	−48.21	−66.34	10.39	−9.67	26.44	11.35	22.51	−0.85	103.85	26.19	−26.77	−36.86	3.46	−5.17
CP65×MU1	−39.06	−55.99	−11.61	−32.11	−12.55	−20.75	−2.37	−6.93	0.26	−0.03	1.45	−15.41	−34.16	−36.51	−36.64	−44.23	−3.39	−8.53
CP65×JG	88.72	73.78	71.13	66.67	−0.76	−2.95	−12.09	−12.09	−11.33	−11.33	−8.18	−8.18	−51.61	−64.29	−24.39	−24.39	−9.60	−10.20
CP65×JJ24	97.78	82.56	123.56	118.01	−4.58	−8.36	−10.20	−12.48	−10.99	−16.65	−11.53	−22.32	−51.81	−52.38	30.13	9.91	−1.46	−4.27
CP65×CP57	−80.85	−85.83	−74.23	−83.03	−26.67	−33.12	−14.66	−14.76	−10.10	−11.77	−60.56	−67.12	59.47	−7.74	−24.84	−27.56	0.24	−15.67
CP47×TD865	−27.49	−29.13	−13.12	−28.45	−8.21	−15.97	−18.24	−18.69	−14.95	−18.96	−34.13	−41.69	−31.62	−54.02	−34.43	−39.19	−7.66	−12.04
CP47×MRSL	−59.38	−69.07	−48.22	−48.62	−41.25	−60.54	−28.02	−35.63	−26.09	−30.17	−28.48	−33.08	119.63	35.06	−37.76	−49.63	−8.63	−20.16
CP47×MU1	0.11	−7.70	95.20	77.56	−5.60	−8.90	−2.85	−8.16	−7.03	−13.97	−25.29	−27.53	−15.15	−19.54	−18.78	−33.01	−7.35	−7.54
CP47×JG	−66.88	−76.10	−66.70	−75.64	−11.73	−19.01	−3.16	−12.50	−2.85	−9.86	−2.21	−16.39	−7.24	−32.30	−4.65	−11.61	−4.12	−8.47
CP47×JJ24	37.99	−0.27	49.65	9.42	0.90	−8.97	2.52	−5.15	1.45	−11.38	−14.90	−17.50	−5.33	−8.05	−35.00	−48.37	−3.98	−11.37
CP47×CP57	7.93	3.16	12.15	−3.46	−3.17	−16.74	1.11	−8.53	−8.12	−16.21	−5.04	−7.88	131.54	33.33	−2.83	−12.94	16.41	−6.15
TD865×MRSL	−48.81	−61.63	−26.14	−38.79	−30.07	−50.59	14.64	2.02	10.68	−0.07	−10.27	−15.46	90.00	58.33	−37.53	−46.16	−1.18	−17.15
TD865×MU1	46.41	32.19	154.50	127.76	−13.87	−18.45	2.10	−2.98	−0.98	−3.98	−30.33	−36.62	70.37	17.95	−3.32	−14.93	−14.00	−17.92
TD865×JG	6.65	−21.92	88.34	61.63	−20.75	−32.87	−6.49	−15.08	−8.75	−11.26	−16.58	−35.47	175.71	141.25	−28.43	−28.47	−18.53	−25.74
TD865×JJ24	−43.94	−58.89	−25.01	−35.72	−20.03	−33.29	−0.30	−7.28	−0.60	−9.31	−33.54	−42.73	−30.80	−52.74	−7.40	−21.83	4.18	−8.03
TD865×CP57	−51.42	−54.57	−51.48	−64.41	−14.28	−31.50	0.18	−8.92	0.64	−3.90	−0.65	−9.61	4.17	−25.00	−18.57	−21.56	−4.66	−25.91
MRSL×MU1	−1.47	−20.21	49.92	37.34	−41.14	−59.70	9.83	−6.52	25.84	10.54	16.46	12.20	104.08	28.21	−16.25	−18.24	−3.31	−15.66
MRSL×JG	13.33	−30.20	68.97	24.27	−45.09	−64.67	17.16	−4.13	36.98	20.63	33.59	8.12	311.67	208.75	−22.84	−33.47	−8.10	−16.27
MRSL×JJ24	−12.22	−45.88	73.11	27.20	−45.77	−65.40	−19.55	−32.80	−16.81	−30.76	−19.89	−27.17	45.10	−9.76	−5.69	−7.98	19.56	12.61
MRSL×CP57	−48.46	−59.45	−29.33	−39.57	−48.87	−68.12	−43.84	−54.00	−36.30	−44.81	−55.81	−57.43	303.61	235.00	−31.54	−39.04	4.97	−4.53
MU1×JG	−73.95	−82.10	−75.40	−80.74	22.99	9.25	−19.46	−23.22	−11.60	−11.86	−49.23	−57.67	−2.64	−26.36	−2.59	−14.25	−4.51	−9.02
MU1×JJ24	65.97	14.19	76.51	38.06	1.86	−10.97	2.13	−0.17	11.42	4.63	−40.90	−44.36	0.99	−1.47	12.94	7.63	−2.97	−10.61
MU1×CP57	−69.81	−70.93	−46.73	−57.62	−37.54	−47.87	1.26	−3.36	3.59	1.95	3.13	3.13	206.91	79.42	−34.04	−39.97	4.28	−16.06
JG×JJ24	122.27	121.67	147.58	147.26	15.11	12.99	2.64	0.03	4.00	−2.61	0.16	−12.05	18.85	−11.59	−8.96	−23.11	3.40	−0.20
JG×CP57	−91.83	−94.26	−91.39	−94.25	−12.34	−18.39	−5.42	−5.54	−5.03	−6.80	−1.90	−18.21	195.02	96.19	−5.81	−9.22	0.17	−16.19
JJ24×CP57	−30.77	−51.33	−46.28	−64.18	44.70	37.09	3.80	1.29	2.86	−1.93	1.49	−4.46	12.04	−34.96	−44.25	−51.42	2.03	−12.06

121.67%. A total of thirteen hybrids had a positive percentage of MH and BPH. Among these hybrids, the maximum heterosis for yield was obtained by JG×JJ24, with values of 122.27 and 121.67% for MH y BPH, respectively. These results were higher than those found by Kaczmarska *et al.* (2016), who reported better-parent heterosis (BPH) of strawberry as high as 28% in crosses derived from a top-cross-mating. For number of berries per plant, positive MH was detected on 15 hybrid families with magnitudes ranging from 12.15 to 154.50%, while 14 F_1 families exhibited positive BPH. The crosses TD865×MU1 and JG×JJ24 had the highest percentage of heterosis. For berry weight, MH ranged from −48.87 to 44.70% whereas BPH varied from −68.12 to 37.09%. In both estimations of heterosis, the hybrids JJ24×CP57, MU1×JG, and JG×JJ24 exhibited the highest heterosis percentages for this trait. In terms of berry length, 12 and 3 crosses reflected the highest positive values of MH and BPH, respectively, which had values that ranged from 0.18 to 17.16 % for MH and 0.03 to 2.02% for BPH. In berry width, BPH varied from −44.81 to 20.63 and MH ranged from −36.30 to 36.98. Among the F_1 hybrids, the highest heterosis was recorded by MRSL×JG. Concerning number of drupelets per berry, few hybrid families exhibited MH and BPH positive values, specifically, seven families for MH and three families for BPH. Among the hybrids, MRSL×JG and MRSL×MU1 were the best crosses with the highest value of heterosis for both cases. In number of canes per plant, 68% of the hybrids displayed positive MH whereas 43% of hybrids exhibited positive values for BPH. For plant height, the heterosis was only found on two families (CP65×JJ24 and MU1×JJ24). In terms of soluble solids content, only one hybrid family showed a positive BPH while ten families exhibited MH positive values. Considering all hybrids, MRSL×JG was the best cross with the highest heterosis for soluble solids content with values ranged from 12.61 to 19.56%. Such results are in agreement with Harbut *et al.* (2009), who evaluated 29 genotypes including 15 cultivars and 14 hybrids. They found that some strawberry hybrids had higher values of fruit weight and others vegetative traits, that their respective parents, indicating that heterosis were present for those traits.

Significant correlations ($P<0.05$) were observed among heterosis values of F_1 progeny for some traits (Figure 1). Almost all detected correlations were similar for both MH and BPH. The yield is a complex trait which is highly influenced by the environment and hence indirect selection through component traits would be an advisable strategy to increase the efficiency of selection (Acquaah, 2007). The yield per plant was only positively correlated ($r=0.89$ for both heterosis estimations) to number of berries per plant. These results are consistent with Stephens *et al.* (2012), who observed a positive correlation between yield and number of berries per plant, suggesting that yield heterosis is mainly caused by the increased number of berries per plant.

The fruit weight has been considered a primary component in the archived yield in each plant. González (2016) evaluated 42 F_1 sub-families of primocane red raspberry obtained under a partial diallel design. He found that the berry weight showed a poor correlation ($r=0.03$) with the yield per plant. In contrast, Radovich *et al.* (2013) detected a moderate correlation between berry weight and yield. In our study, we found significant correlation between berry weight and berry length ($r_{BPH}=0.41$), and number of canes per plant ($r_{MH}=-0.59$ and $r_{BPH}=-0.56$), but a non-significant association with yield,

	Yield/ plant	Number of berries per plant	Berry weight	Berry length	Berry width	Number of drupelets per berry	Number of canes per plant	Plant height	Total soluble solids
Yield/plant	1	0.89***	0.37	0.26	0.09	0.20	−0.25	0.30	0.24
Number of berries per plant	0.89***	1	0.11	0.20	0.14	0.15	−0.09	0.31	0.19
Berry weight	0.25	−0.01	1	0.41*	0.04	−0.08	−0.56**	0.12	0.06
Berry length	0.24	0.27	0.10	1	0.81***	0.51**	−0.29	0.07	−0.24
Berry width	0.20	0.25	−0.09	0.91***	1	0.58**	−0.01	0.02	−0.32
Number of drupelets per berry	0.20	0.20	−0.12	0.69***	0.70***	1	0.06	−0.16	−0.02
Number of canes per plant	−0.28	−0.13	−0.59**	−0.03	0.12	0.19	1	−0.20	−0.21
Plant height	0.35	0.32	0.15	0.03	0.01	−0.14	−0.21	1	0.20
Total soluble solids	−0.14	−0.21	−0.11	−0.06	−0.08	0.04	0.10	0.16	1

Figure. 1. Significant correlation coefficients among mid-parent (MP, lower diagonal) and better-parent (upper diagonal) heterosis values for yield and its components. *, **, *** indicate significant difference at $P<0.05$, 0.01 and 0.001, respectively.

suggesting the association between yield and yield-components is complex and depends on genetic diversity degree and population type (floricane or primocane).

Berry length was positively correlated to berry width ($r_{MH}=0.91$ and $r_{BPH}=0.81$) and number of drupelets per berry ($r_{MH}=0.69$ and $r_{BPH}=0.51$). Berry width also exhibited significant positive correlation with the number of drupelets per berry ($r_{MH}=0.70$ and $r_{BPH}=0.58$). All previous results suggest that the heterosis for size fruit-related traits is influenced for the level of heterosis exhibited in the number of drupelets per berry. Similarly, Radovich *et al.* (2013) found that fruit size was positively affected by number of fruiting laterals and drupelets per berry. In addition, such positive association has also been reported in blackberry (Strik *et al.*, 1996), where cultivars presenting a high number of drupelets also exhibited a large fruit. Finally, we found a significant correlation between number of drupelets per berry and number of berries per plant ($r_{BPH}=0.38$); however, such association was only detected in the better parent heterosis values.

CONCLUSION

Some raspberry families out-yielded 'Autumn Bliss' and simultaneously showed significant heterosis for yield and other yield components. Specifically, the progeny derived from parents MU1 and TD865 had good agronomic performance and positive heterosis for fruit size-related traits, soluble solids content and yield. Concerning the association between yield-components and yield heterosis, the number of berries per plant was the more important yield-component affecting the yield heterosis expression.

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AGRO PRODUCTIVIDAD

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Commercial dynamics of mexican tomato in the framework of the USMCA: an analysis of trade with the united states using the gravity model

Gómez-Gómez, Alma Alicia[2]; **Hernández-Mendoza, Natividad**[2]; **Luquez-Gaitan, Carlos Ernesto**[1,2*]

[1] Universidad Politécnica de Texcoco.
[2] Universidad Autónoma Chapingo. Economía Agrícola.
* Correspondence: carlosluquezgaitan@gmail.com

ABSTRACT

Objective: Within the framework of the treaty between Mexico, the United States and Canada (USMCA), the objective of this study is to provide a description through econometric methods of the variables that influence tomato trade, in addition to describing the commercial dynamics of the sector in both Mexico and the United States.

Design/Methodology/Approach: A gravity model was applied to investigate and evaluate the role of some of the main economic and geographic variables as determinants of Mexican trade flows.

Results: The results show that the most important variables are the US gross national income *per capita* (GNI_{PC}), as well as the US *per capita* production and consumption volumes calculated from apparent national consumption (ANC). It was also found that the variable GNI_{PC} is better to determine the model than the gross domestic product *per capita* (GDP_{PC}), due to the qualities of the variables.

Limitations/Implications: Statistical records for the period 1994 to 2020 were taken into account, considering all varieties of tomato produced and exported.

Findings/Conclusions: Regarding income, the variable with the best fit in the model was in GNI_{PC}, which was adopted in the World Bank's current way of classifying countries by income, variables such as consumption and production behaved in a typical way increasing and decreasing the volume traded. Tomato (*Lycopersicon esculentum* Mill.) is one of the most competitive and profitable agricultural products in Mexico.

Keywords: Agricultural Econometrics, International Agricultural Trade, Agricultural Competitiveness.

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INTRODUCTION

Mexico is the main supplier of tomato worldwide, with an international market share of 25.11% of the value of world exports (SAGARPA, 2017). Tomato (*Lycopersicon esculentum* Mill.) is one of the most consumed vegetables in the world (Castillo, Moreno-Pérez, & Cruz-Arellanes, 2009). It is one of the most important horticultural species produced in Mexico, due to its importance not only as a generator of foreign currency, but also because of the high economic spillover it generates (Reyes, 2014).

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According to SIAP (2017), production was 3.35 billion tons. Since 1994, Mexico has a free trade agreement with the United States and Canada that allows it to have preferential treatment in terms of international trade with both nations; the agreement has had several modernization processes and in its latest version was given the name United States-Mexico-Canada Agreement or USMCA.

The USMCA is the new trade agreement between Mexico, the United States and Canada that replaces the North American Free Trade Agreement (NAFTA). On November 30, 2018, the three nations signed a new agreement to begin the process of updating the Treaty within the framework of the G-20, held in Buenos Aires (FORBES, 2020); this update did not modify what was previously established for tomato trade in NAFTA.

The red tomato or tomato taken into account for analysis in this study, according to the Committee for Tariff Classification (CCA-CAAAREM) (2021), is represented by tariff fraction 070200 and refers to fresh or refrigerated tomatoes; it is not subject to tariffs for exports or imports, and its import is not subject to payment of the value-added tax (VAT). The SIAVI (2021) specifies that there is no applicable tariff for imports from the United States and Canada.

NAFTA established a trade liberalization regime of up to 5 and 10 years for some horticultural products, which included the establishment of tariffs and safeguard fees during the period of total trade openness. In the case of tomato, several periods were established to consider seasonal factors. From November 15 to February 28 (Period 1), the tax relief period was established for 10 years with a tariff of 3.3 US dollar cents per kilogram and a safeguard fee of 172.3 thousand tons, which would grow at a compound annual rate of 3%. From March 1 to July 14 (Period 2) the tariff has been zero since the beginning of 2004, with a rate of 4.6 US dollar cents per kilogram and a safeguard fee of 165.5 thousand tons, which will also increase 3% each year. A 5-year tax relief period was established for the periods from July 15 to August 31 (Period 3) and from September 1 to November 14 (Period 4), with tariffs of 3.3 and 4.6 US dollar cents per kilogram, respectively; in this case, no safeguard fee was established (Bay, DeFrehn & Fox, 1994). According to Garcia, Williams and Malaga (2005), such tariffs and safeguards were a consequence of the protection granted to Florida producers by the U.S. Government.

During the 1999-2001 period in the North American market had a consumption and production of 4.37 and 4.31 million tons, respectively. The region was almost self-sufficient, since 98.3% of the demand was supplied by NAFTA countries, and only 1.7% came from the rest of the world. Of North America's tomato production, 99.7% was consumed in the same region. In the same period, consumption was 2.48, 1.64 and 0.255 million tons for the US, Mexico and Canada, respectively, while production was 1.89, 2.24 and 0.18 million tons (Garcia *et al.*, 2005). According to SAGARPA (2017), during 2016 Mexican tomato covered 90.67% of imports in the United States and 65.31% in Canada.

Currently, 100% of national requirements are met with domestic production, and world imports have increased 34.41% in the last decade, which has generated an increase in Mexican exports mainly destined to the United States (SAGARPA, 2017). Since there is self-sufficiency in the region, a considerable difference between Mexican production and consumption, it is logical to assume that the unsatisfied demand in the United States is supplied mainly by Mexican exports; the geographic position could be the most important factor to explain the success of exports, and Mexico's favorable climatic factors should also be taken into account, the seasonality of exports shows that between January and April are the months of greatest commercial flow abroad as shown in Figure 1. Consequently, from the above, an analysis is suggested through the gravity model.

As stated by CEPAL (2017): "The gravity model has been used to analyze the determinants of bilateral trade relations since its introduction by Tinbergen (1962). The basic idea is to approximate trade between two countries according to a Newtonian gravity rule, where the degree of trade is directly related to the size of the trading economies and inversely related to the distance that separates them. In the 1960s and 1970s it received much criticism regarding its foundation, since the models that explained trade patterns (Ricardo and H-O) were not able to explain the relationship. However, from the empirical point of view, the explanatory power and its robustness were unquestionable. Krugman (1997) referred to the gravity equations as examples of 'social physics', the relatively few empirical regularities that characterize social interactions". Because of this and Mexico's trade in the USMCA, it is appropriate to develop an analysis through the gravity model.

Due to the importance of tomato farming in Mexico's primary sector and in the global market, the objective of this study is to measure the effects of the USMCA on the growth experienced by fresh tomato exports from Mexico to the United States, which is the main importer worldwide, during the period 1994-2020, as well as to determine the effects of the total elimination of tariffs under NAFTA on Mexico's trade dynamics.

Due to the low level of tariffs agreed to in NAFTA and their subsequent elimination, the hypothesis put forward is that the effects of tariff relief have been moderate on the growth of Mexico-US trade. That is, it supports the claim that the absolute growth experienced by Mexico's exports to the United States during the period from 1994 to 2020 is due to non-NAFTA factors, as proposed by García *et al.* (2005).

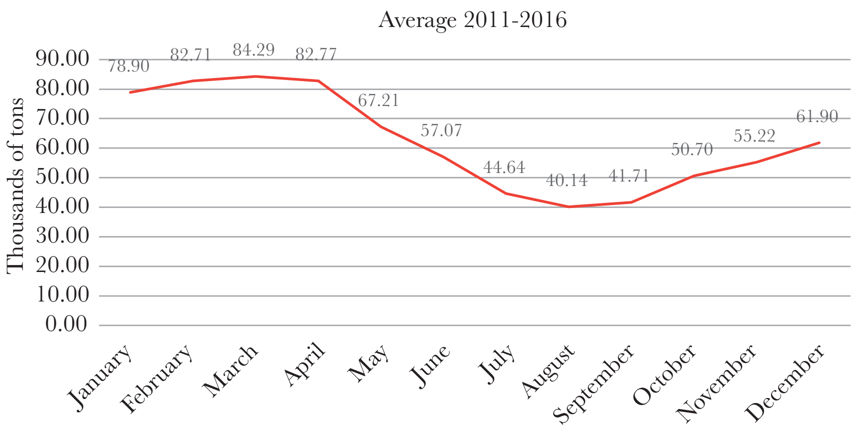

Figure 1. Seasonality of tomato exports.
Source: SAGARPA, 2017.

MATERIALS AND METHODS

The empirical evidence for the gravity equation in international trade is strong. The contribution made by Krugman (1980) was motivated in part by the empirical regularity of the gravity equation. His model explains how, in the aggregate, trade flows are proportional to country size and inversely related to trade barriers.

Distance and economic size have been remarkably stable variables over time, in different countries, and using several econometric methods. Existing theoretical models can easily explain the role of economic size in trade flow participation, but none explain the role of distance. To the extent that distance represents trade barriers, their model can also explain why distance has a negative impact on trade flows in general, but has nothing more to say about the specific role of distance (Chaney, 2011).

The gravity equation is very successful in explaining international trade empirically (Sá Porto, 2000). The gravity model represents a kind of spatial interaction model and can be used to calculate the number of interactions between two countries. The fundamental idea behind spatial interaction models is that the degree of interaction between two countries is a function of the degrees of concentration of people or things in the two countries, and a measure of the distance separating these countries (Cardoso, Rasetti, Giampietri, Finco & Shikida, 2017).

A time series gravity model was used to analyze the dynamics of trade between Mexico and the United States, member countries of the USMCA during the period from 1994 to 2020, considering data related to the trade of fresh tomato with tariff fraction 0702000.

Data documentation was carried out mainly through 5 sources: the Agrifood and Fishing Information Service (*Servicio de Información Agroalimentaria y Pesquera*, SIAP, 2021) with its Agrifood Information Consultation System (*Sistema de Información Agroalimentaria de Consulta*, SIACON NG, 2021) tool; the TRADEMAP tool that was developed by the International Trade Center of UNCTAD/WTO (INTRACEN, 2006); the FAOSTAT databank from Food and Agriculture Organization of the United Nations (FAO) which provides free access to food and agriculture data for more than 245 countries and 35 regions, from 1961 to the most recent year available (FAO, 2021); the United States Foreign Agricultural Service (USDA, 2021); and the World Bank's Open Access Data (World Bank, 2021).

The gravity model estimated to analyze trade dynamics has the following form:

$$Vcommercial_{Mx-USA}=\alpha_0+\alpha_1 GNIpc_{USA}+\alpha_2 GNIpc_{MX}+\alpha_3 PRODpc_{USA}+\alpha_4 CONSpc_{USA}+v_{ij}$$

Where: $Vcommercial_{Mx-USA}$=Is the commercial volume of US plus Mexican tomato expressed in tons. $GNIpc_{USA}$=Gross national income at real *per capita* prices for the United States. $GNIpc_{MX}$=Gross national income at real *per capita* prices for Mexico. $PRODpc_{USA}$=United States *per capita* tomato production. $CONSpc_{USA}$=Apparent national *per capita* consumption of US tomato. v_{ij}=Estimation error.

An ordinary least squares (OLS) regression was estimated to make an initial approximation of the results that were intended to be found through the proposed model. Signs of inconsistency were found, denoted by the values that were estimated for the t-Student test, which was designed to examine the differences between two independent and small samples that have normal distribution and homogeneity in their variances (Sánchez, 2015), which translates into the loss of the optimality of the OLS.

In this first estimation it was found that one of the main problems with the model was the serial autocorrelation and multicollinearity of the independent variables. This implies that the variances and covariances matrix of the disturbances present non-zero values in the elements outside the main diagonal (Gujarati, 2004; Griffiths & Judge, 1993), and not due to the estimated values of the t-test, but rather because it is an annual time series with 27 observations.

When the Durbin-Watson test was calculated and serial correlation was evidenced in an OLS regression, the Durbin-Watson test contrasts the first-order autocorrelation under the linear regression model with autocorrelation and tests whether the differences between consecutively observed residuals are very large or small (Universidade de Vigo, 2019), which implies that, according to Roldán Rosales (2021):

- The OLS estimator is still linear and unbiased, but it is not of minimum variance and there is another more efficient linear estimator.
- The variances and covariances of the OLS estimators are biased.
- The confidence intervals and the usual statistics for hypothesis testing are not adequate.
- The R^2 statistic is biased.

Therefore, the use of OLS to estimate the effects of the trade dynamics was initially ruled out. In this case, to correct the autocorrelation problem in the linear regression model it is necessary to perform a transformation of the model leading to the use of the generalized least squares (GLS) technique in the Cochrane-Orcutt or Prais-Winsten procedures (De Lira Arenas, 2011).

In addition, the use of robust estimators was applied in the definitive estimation, because it eliminates the loss of optimality of the OLS in face of two possible disturbances that normally occur in economic series in some abnormal periods, when the errors are not normal and the model may fail to be fulfilled (Yohai, 1984). The application of these two methods together could be expressed as a robust estimation in autoregressive processes, and showed improvement in the results of the hypothesis tests. All estimations were performed using STATA version 12 econometric software.

RESULTS

To decide which model was the most appropriate for analyzing Mexican tomato trade under the USMCA, this study applied the Durbin-Watson test to an ordinary least squares regression and resulted in a value of 0.93. The test showed the presence of first-order autocorrelation. This problem was solved by considering the minimization of standard errors through robust estimators in conjunction with the application of the Prais-Winsten methodology, and it was the best solution to estimate this gravity model which transformed the Durbin Watson test from 0.93 to 1.77; the results can be seen in Table 1.

Table 1. Results of the Gravity Model.

Dependent variable=Trade MEX-USA	Coefficient	Standard error	T-student	P-value
EE. UU. GNI *per capita*	4.95	2.71	1.82	0.082
Mexico GNI *per capita*	9.48	12.3	0.77	0.449
Production *per capita* EE. UU.	−261681.5	20375.97	−12.82	0
ANC *per capita* EE. UU.	264138.2	20560.25	12.85	0
Constant	−187697.8	101372.6	−1.85	0.078
R^2 0.94		Durbin-Watson	F(4, 22)=151.53	
		1.77	Prob>F=0.000	

Source: Prepared by the authors with data from World Bank, Trade Map, SIACON, INTRACEN, FAOSTAT and USDA.

The F statistic defines the hypothesis test that all coefficients are simultaneously zero; that is, all explanatory values together have no impact on the regression. Given that the calculated F value of approximately 151.53 is highly significant and the (Prob>F=0.000), it means that the variation in the dependent variable can be explained by the explanatory variables, with the coefficients in the model being different from zero.

The high R^2 value of 0.94 can be explained by the fact that the Mexican trade under analysis contemplates a long time series of 27 periods; variables such as a country's total production are variables that move very little during a short period of time, and the behavior of one period is highly influenced by the behavior exhibited in the previous period.

The use of Gross National Income (GNI) *per capita* instead of Gross Domestic Product (GDP) *per capita* is due to the methodology by Serajuddin & Hamadeh (2020) of the World Bank, where they use GNI to measure the income levels of countries and is part of the contribution to the gravity model methodology of this study.

Among the explanatory variables considered by the time series gravity model, the Mexican GNI *per capita*, which represents the purchasing power of the population, is not significant for tomato trade. One possible reason could be the existence of a relatively large domestic market; accordingly, Cardoso *et al.* (2017) argue that a home bias effect, such as local distribution networks, may play a more important role in trade compared to the GDP.

In contrast, the US GNI *per capita*, being a proxy for the magnitude of wealth, was a significant variable at 93% confidence. In particular, if "US GNI increased by 1%, US trade increased by 4.95 tons. According to this, it is expected that the higher the GDP of exporting countries, the greater their capacity to supply the consumption needs of importing countries (Cardoso *et al.*, 2017).

Regarding the consumption variable, representing the potential US tomato market, it showed a direct relationship with trade. The results indicate that increases in US consumption make US-Mexico trade increase. A possible reason is that tomato varieties produced in Mexico are highly demanded by US consumers, and because of this, trade in tomato produced in Mexico increases when US consumption increases, probably because it satisfies specific consumer interests and needs absolutely.

In relation to the production variable, it showed an inverse relationship with trade. Results indicate that increases in US production decrease trade between the US and Mexico. A possible reason is that tomato varieties produced in Mexico are less desirable than those produced in the US, so trade of tomato produced in Mexico decreases when US production increases. Finally, the distance and proximity variables are fixed effects, which is to say that they do not vary over time and would only generate collinearity; therefore, these variables were omitted from the model.

CONCLUSIONS

In order to determine the trade dynamics of the USMCA member countries on an annual basis, it is pertinent to take into account the entire series of data available since 1994, which generates sufficient data to perform procedures that correct problems generated by time series analysis. The OLS estimation was biased and lacked optimality due to the presence of first-order serial autocorrelation. This difficulty was solved through the application of the Prais-Winsten methodology in combination with robust estimators to ensure model optimality.

The ANOVA uses the F-test to determine if the variability between means of the groups is greater than the variability of the observations, due to the high calculated value; it can be concluded that not all means are equal, and the model is globally accepted to explain the phenomenon. The model in general presents a high goodness of fit due to its high R^2 statistic, a typical characteristic in time series models. The relationship of the F test and R^2 is fulfilled.

The way in which the economic level of countries can be determined has changed over time, and currently the World Bank is the international organization responsible for providing guidelines on how to classify the income of a nation and uses GNI *per capita*; this indicator gave much better results through the calculated t statistic than the classic GDP *per capita* used in gravity models consulted in the literature.

The GNI *per capita* for the US is accepted at 93% and shows a positive sign, which is in accordance with expectations, while for Mexico the statistic lacks significance and does not show the expected sign; this can be explained by the fact that tomato is a normal basic consumption good in Mexico.

The consumption variable was constructed through US apparent domestic consumption and has a positive influence on trade, which is to say US consumption fosters an increase in Mexican exports. Production showed the expected relationship because if US production increases, it is logical that it discourages imports from Mexico. The model assumes geographic proximity variables, because not changing the data generates collinearity in the regression, reason why the software omits them.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Determining water quality of the lower basin of the Usumacinta River in Tabasco, Mexico

Salcedo-Garduño, Magnolia Gricel[1]; Galaviz-Villa, Itzel[2*]; Pérez-Vázquez, Arturo[1]

[1] Colegio de Posgraduados Campus Veracruz. Carretera Xalapa-Veracruz km 88.5, Tepetates, municipio de Manlio Fabio Altamirano, Veracruz, México. C. P. 91690.

[2] Tecnológico Nacional de México. Instituto Tecnológico de Boca del Río. Carretera Veracruz-Córdoba km 12, municipio de Boca del Río, Veracruz, México. C. P. 94290.

* Correspondence: itzelgalaviz@bdelrio.tecnm.mx

ABSTRACT

Objective: To determine the water quality of the lower basin of the Usumacinta River (Tabasco, Mexico), based on its physicochemical and microbiological characteristics.

Design/methodology/approach: There were taken 11 monthly samplings of surface water during the rainy season, from June to December, 2017 and in the dry season, from April to May, 2018, in three different sites. The parameters of temperature, Hydrogen potential (pH), Electrical Conductivity (EC), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), total phosphorus (TP) and Nitrogen nitrate (N-nitrate) were evaluated. Significant statistical differences ($p \leq 0.05$) between seasons and among sites were determined.

Results: The results obtained were compared with Mexican regulations and Ecological Criteria for water quality. The parameters such as EC, TDS, TSS and TP showed substantial differences among seasons. Values for temperature, pH, EC and N-nitrate, did not exceed the maximum permissible limits for the use of irrigation water, urban public use and protection of aquatic life. However, the presence of *E. coli* and total coliforms from 530 to >24,196 MPN 100 mL^{-1} were recorded throughout the study period.

Findings/conclusions: Parameters under study did not show significant differences among sites. The rainy season had an important effect on the increase in concentrations of TSS, TP, N-nitrate and total coliforms. Also, maximum concentrations were recorded during the study period in the sampling sites of Boca del Cerro (R1) and Puente Chablé (R2).

Keywords: Diffuse contamination, anthropogenic pollution, physicochemical and microbiological parameters, protection of aquatic life, Usumacinta River.

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INTRODUCTION

The basin of the Usumacinta River in the state of Tabasco, Mexico, is one of the regions with great biodiversity and importance in the Maya Biosphere Reserve, in Mexico. In addition, it is classified as one of the water bodies with high vulnerability due to the impact of anthropogenic activities (Cotler *et al.*, 2010; López, 2011). Some sources and activities that contribute to the contamination of the environment and the deterioration of the water quality of Rivers, lakes, aquifers and coastal waters in various parts of the world

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are: human settlements, percolation of landfill leachates, diffuse pollution, sediments, pathogens, and metals (Mateo-Sagasta *et al.*, 2017; FAO, 2018). The increase of nutrients such as nitrogen and phosphorus in aquatic systems produce the eutrophication of water bodies with algal blooms causing these conditions mortality of aquatic organisms (Elser, 2012). The objective of this study was to evaluate the water quality of the lower basin of the Usumacinta River based on the determination of its physicochemical and microbiological characteristics according to the Official Mexican Norm NOM-001-SEMARNAT-1996 and the Ecological Criteria of water quality CE-CCA-001-1989.

MATERIALS Y METHODS

Study area

This research was carried out in the lower basin of the Usumacinta River, Tabasco, in the municipalities of Tenosique de Pino Suárez, Emiliano Zapata and Jonuta (Figure 1). The Usumacinta River basin has 106,000 km^2 of territory located between the states of Chiapas, Tabasco and Campeche in Mexico. Its water presents currents from south to north, flowing into the Gulf of Mexico with an approximate load of 105,200 million m^3 of water per year. According to García (2018), the sites of Puente Boca del Cerro (R1), Puente Chablé (R2) and Puente Jonuta (R3) presented maximum flow during the rainy season in October, 2017. These were 3278.3, 4901.24 and 3457.28 $m^3\ s^{-1}$ respectively. While lower values were recorded during the dry season in April, 2018, these were 376.3, 827.34 and 444.87 $m^3\ s^{-1}$ for each sampling site, respectively.

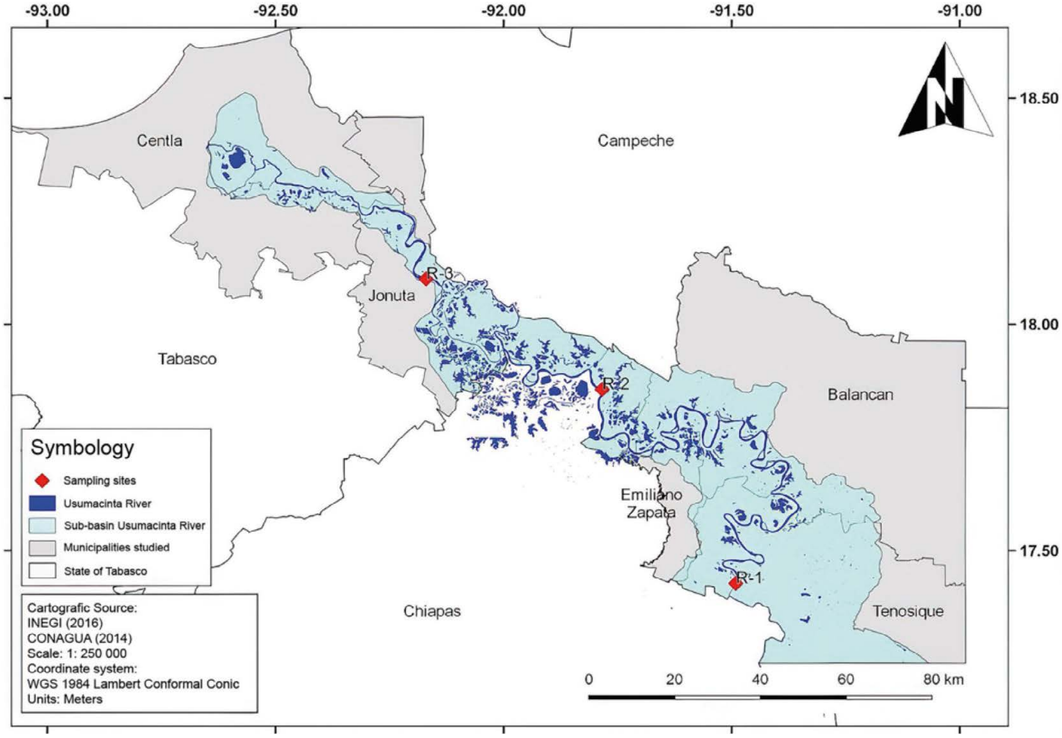

Figure 1. Sampling sites in the lower basin of the Usumacinta River in Tabasco, Mexico. R1 (Puente Boca del Cerro) 17.427387° N, 91.490958° W; R2 (Puente Chablé) 17.856130° N, 91.784002° W; R3 (Puente Jonuta) 18.100300° N, 92.143470° W.

Water sampling

Three sampling sites were selected, which are located in: Tenosique de Pino Suárez (R1, Puente Boca del Cerro), Emiliano Zapata (R2, Puente Chablé), and Jonuta (R3, Puente Jonuta). The samplings were made monthly, based on the procedure NMX-AA-003-1980; from June to December, 2017 and from April to May, 2018. According to the hydrograph of CONAGUA (2017), it was taking into account the rainy season, in the period from June to October, 2017 and the dry season, from November, 2017 to May, 2018. Temperature, EC and pH measurements were made *in situ*, with two HATCH multi-parameter probes (sesIONTM+Portable Meter and sesIONTM+MM150).

The samples were collected between 09:00 and 16:00 h at a depth of 20 cm below the water mirror. The sample volume to determine TDS (Total dissolved solids), TSS (Total suspended solids), N-nitrate $\left(N\text{-}NO_3^-\right)$ and total phosphorus (TP) was 500 mL, which was collected in transparent polyethylene bottles. For the bacteriological analysis, sterile bags of 120 mL were used. All water samples were refrigerated at 4° C. The reading of physicochemical parameters *in situ* was carried out under procedures of test methods established in the standards NMX-AA-007-SCFI-2013 and NMX-AA-008-SCFI-2016. The methods for the determination of each of the parameters were: TDS and TSS (NMX-AA-034-SCFI-2015), N-nitrate (NMX-AA-079-SCFI-2001/US EPA-353.2-1993) and total phosphorus (NMX-AA-029-SCFI-2001/US EPA-365.1-1993). The determination of coliforms was performed by the most probable number method per 100 mL (MPN 100 mL^{-1}) with the Colisure and Quanti-Tray Chromogenic Substrate Laboratory/ Quanty-Tray/2000.

Statistical analysis

An analysis of descriptive statistics was made to the results of the concentrations, applying a normality test of Shapiro-Wilk. To determine significant statistical differences between seasons, the following tests were used: U Mann-Whitney ($p \leq 0.05$) and t-student ($p \leq 0.05$); and the Kruskal-Wallis test ($p \leq 0.05$) to determine differences among sites. The analyzes were performed using the IBM® SPSS® Statistics Version 25 program.

Maximum permissible limits (MPL)

To determine water quality, the MPL of the official Mexican standard (NOM-001-SEMARNAT-1996) and the ecological criteria of water quality (CE-CCA-001-1989) were used. This, in the areas of use of agricultural irrigation, urban public use and protection of aquatic life. The parameters that are not included in any of the previous references were compared individually with reference values obtained in other research works.

RESULTS AND DISCUSSION

In this study, the temperature varied from 25.6 to 30.7 °C in the rainy season, while in the dry season it oscillated between 22.96 and 33.88 °C in R1 and R3, respectively (Table 1).

Table 1. Contrast of the Maximum Permissible Limits (MPL) according to NOM-001-SEMARNAT-1996, the Ecological Criteria of Water Quality CE-CCA-001/89, with regards to the concentration of the parameters analyzed for the lower basin of the Usumacinta River, Tabasco, Mexico.

MPL	**T (°C)**	**pH**	**EC (μS cm^{-1})**	**N-NO_3^- (mg L^{-1})**	**TP (mg L^{-1})**	**TDS (mg L^{-1})**	**TSS (mg L^{-1})**	**TC (MPN 10 mL^{-1})**	**FC (MPN 100 mL^{-1})**
NOM-001-SEMARNAT-1996	40[1,2,3]	n/s	n/s	40[1,2] 15[3]	20[1,2] 5[3]	n/s	150[1], 75[2], 40[3]	1000	n/s
CE-CCA-001/89	n/s	4,5-9[1]	1000	n/s	0.1[3]	500[1]	50[1]	1000[1]	1000[1]
Rainy season									
Maximum	30.7	8.93	51.40	0.638	**0.461**	335	**300**	**14 136**	Presence
Minimum	25.6	7.50	31.96	0.0015	0.0334	198	**70**	571	-
Dry season									
Maximum	33.88	8.13	83.83	0.459	**0.65**	**616**	**73**	**>2419.6**	Presence
Minimum	22.96	7.68	45.66	0.091	0.022	330	0	530	-

MPL: Maximum permissible limits; T °C: Temperature; pH: Hydrogen potential; EC: Electrical conductivity; N-NO_3^- : Nitrogen nitrate; TP: Total phosphorus; TDS: Total dissolved solids; TSS: Total suspended solids; TC: Total coliforms; FC: Fecal coliforms; n/s: not specified. Bold letters are data above the MPL. [1]For agricultural irrigation; [2]Urban public use; [3]Protection of freshwater aquatic life.

Due to the fact that the state of Tabasco has a humid and a dry period, with abundant rainfall almost all year round, surface water registers significant variations in temperature, being April the driest month in the state of Tabasco, with higher (environmental) temperatures. However, in this study the maximum temperatures recorded were in May with 33.8 °C and followed by April with 31.2 °C.

The pH values during the rainy season varied from 7.50 to 8.93 in R1 and R2, while in the dry season it varied from 7.68 to 8.13 in R2 and R3, respectively. CE-CCA-001/89 considers water suitable for agricultural irrigation to all those that maintain a pH between 4.5 to 9.0 (Table 1). The pH values were found within the range of 6 to 9.1, similar to those reported by Ramos-Herrera *et al.* (2012), in the Usumacinta River.

The EC had a range of 31.96 to 51.40 μS cm^{-1} during the rainy season at R3. In the dry season, the EC ranged from 45.66 to 83.83 μS cm^{-1} at R2. According to CE-CCA-001/89, waters suitable for agricultural irrigation are all those that maintain a value below 1000 μS cm^{-1} (Table 1).

When EC values lower than 250 μS cm^{-1} are recorded in surface water, this is considered excellent for agricultural use (Guzmán-Quintero *et al.*, 2007), as recorded in this investigation. EC values in this study showed a tendency to increase during the dry months. According to López (2011), the EC is related to salts dissolved in water. This author suggests that the behavior of the EC values in the Usumacinta River are directly related to the concentrations of TDS, and to the temperature increase in surface water (Solís-Garza *et al.*, 2011).

TDS ranged from 198 to 335 mg L^{-1} at sites R2 and R3 during the rainy season and from 330 to 616 mg L^{-1} during the low season at R1. The average values in the rainy season were 265.7 mg L^{-1} and in the low season 469.16 mg L^{-1}. CE-CCA-001/89 indicate that water for agricultural use should contain less than 500 mg L^{-1}. In April and

May 2018, all the sites exceeded the MPL, with the exception of R3 in the low season, which was 499 mg L^{-1} (Table 1). TDS in this study showed an increasing tendency pattern in the dry season. The elevation of TDS concentration is attributed to the concentration of anions and cations, minerals or contaminants present in the water, since the scarce availability of water hinders the dilution of the solids, which produces turbidity in the water (López, 2011). TSS oscillated between 70 and 300 mg L^{-1} in the rainy period at R3 and R2; while in low tide, values from 0 to 73 mg L^{-1} were recorded at R1 and R2. According to the standard NOM-001-SEMARNAT-1996, the TSS content must be less than 150 mg L^{-1} for agricultural use, less than 75 mg L^{-1} for urban public use and less than 40 mg L^{-1} to preserve aquatic life. The ecological criteria of water quality (CE-CCA-001/89) indicate that for agricultural use the concentration of TSS must be less than 50 mg L^{-1}. The value of the average concentrations recorded for the rainy and low season were 114.27 and 32.16 mg L^{-1}, which indicates that in the rainy season the MPL of the three water use classifications are exceeded. This was also observed in the dry season at the three sites during November and in December at R1. The average values in the rainy season were 114.27 mg L^{-1} and in the dry season 32.16 mg L^{-1} (Table 1). Maximum values of TSS in this study, 300 mg L^{-1}, were lower than those reported by Ramos-Herrera *et al.* (2012), in the Usumacinta River, in the municipalities of Emiliano Zapata and Jonuta. However, the minimum concentrations, 4 and 1 mg L^{-1}, recorded were much lower than those in this study, 13 and 14 mg L^{-1}.

The N-nitrate values recorded in the rainy season ranged from 0.0015 to 0.638 mg L^{-1} at R2; while, in the dry season the values were from 0.091 to 0.459 mg L^{-1} at R3 and R1. According to the norm, surface water with nitrate concentrations lower than 40 mg L^{-1} can be used as irrigation water and for the urban public sector, while waters with concentrations lower than 15 mg L^{-1} are safe for aquatic life, since they do not represent a risk for the species (Table 1). In this study, the registered N-nitrate concentrations were not higher than 0.638 mg L^{-1} during the rainy season, but with a tendency to rise during this season. The highest concentrations were registered at site R2 and R3 during the rainy season, which can be attributed to anthropogenic nitrogen entry from the town of Chablé. This, through waste run-off from clandestine garbage dumps, domestic, as well as effluents from backyard animal farms and application of fertilizers in rainfed crops, located on the banks of the Usumacinta River (Romero *et al.*, 2011). The study area produces mainly cattle, swine and poultry. The crops with the largest area planted and cultivated are corn, sugarcane, oil palm, pumpkin, sorghum, and beans, among others (INEGI 2015). Applying as fertilizer, Urea (46% nitrogen), Triple 20-30-10 (NPK), Triple 17 (NPK) and DAP 18-46-00 (NP), plus irrigation with well water, river and rain (key informants).

The total phosphorus concentration during the rainy season was 0.0334 to 0.4661 mg L^{-1} at R3 and R1, while during the dry season it ranged from 0.022 to 0.65 mg L^{-1} at R3 and R1. In this study, phosphorus concentrations are within the permissible limit according to NOM-001-SEMARNAT-1996. On the other hand, CE-CCA-001/89 indicate that in order to avoid the development of undesirable biological species and to control accelerated eutrophication, total phosphorus values in surface water should not exceed 0.1 mg L^{-1}. However, mean values of 0.105 mg L^{-1} were obtained during the rainy season. Thus, in accordance with CE-CCA-001/89, site R1 exceeded the permissible limits in both seasons (Table 1). The tendency of increase of the registered concentrations of total phosphorus during the rainy season in some places was similar to the study by Musálem-Castillejos *et al.* (2018). The concentrations reported for this study ranged from 0.033 to 0.46 mg L^{-1} during the rainy season, and 0.022 to 0.65 mg L^{-1} in the dry season. The above can be attributed to the hauling of material, dumping and dragging of fertilizers applied to rainfed crops (Romero *et al.*, 2011). In this study the highest concentrations of total phosphorus were observed in the sites with constant presence of human activities. The highest levels of phosphorus, 0.119 and 0.65 mg L^{-1}, were recorded at R1. Considering that the population of Boca del Cerro has inhabitants established on the banks of the river, which would explain why the waste produced by these, plus the runoff, is discharged directly to the Usumacinta. In this study, concentrations of total phosphorus and N-nitrate did not exceed the maximum permissible limits of the norm for agricultural irrigation, urban public use and protection of aquatic life. However, due to the tendency of increasing concentrations of both during the rainy season, there is a condition that leads water systems towards eutrophication during rainy periods, which represents a limitation for their use and a risk for the aquatic life (Romero *et al.*, 2011).

The presence of total coliforms during the rainy season was 571 to 14 136 MPN 100 mL^{-1} at R3 and R1 and at low water levels it ranged from 530 to greater than 24 196 MPN 100 mL^{-1} at R1 and R2. In R3 in September 2017, 8704 MPN 100 mL^{-1} was registered, these concentrations would be close to the MPL (Table 1). The values of the average concentrations were 4076.7 and 4551.2 MPN 100 mL^{-1} in the rainy and dry season respectively. It should be mentioned that the presence of *E. coli* was detected in all the sites and seasons of this study (Table 1). Physicochemical properties of water are related to the presence of coliform bacteria, where their presence and levels are closely related to temperature, pH, suspended solids (Hong *et al.*, 2010), total solids (Qi *et al.*, 2008) and organic and inorganic nutrients in the water; with greater magnitudes in the presence of precipitation, especially in reservoirs close to urban or industrial areas (Hong *et al.*, 2010). For Mateo-Sagasta *et al.* (2017), fecal coliforms are related to intensive grazing and the presence of cattle feedlot pens.

The results of the microbiological analyzes show fecal contamination of the water and presence of total coliforms with a tendency to increase during the rainy season (571 to 14 136 MPN 100 mL^{-1}). This behavior is similar to that recorded by Musálem-Castillejos *et al.* (2018). Thus, variations in coliforms at all sites during the study period may be related to the contribution of organic matter and solids through runoff and soil washing, and points of discharge of: wastewater, animal breeding, agricultural and domestic, that do not present any type of treatment (Romero *et al.*, 2011).

There were not found significant differences in the parameters of temperature, pH, total coliforms and N-nitrate ($p>0.05$). In the observed parameters there were significant differences between seasons for EC, TDS, TSS and total phosphorus ($p<0.05$) with an CI (Confidence Interval) of 95% (Figure 2).

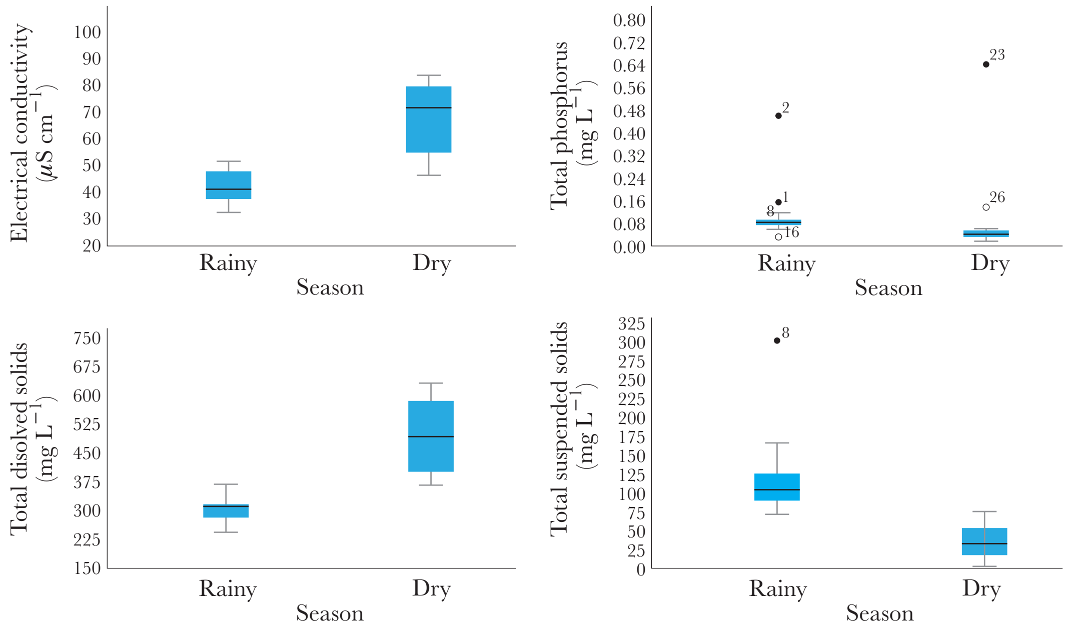

Figure 2. Statistical analysis (95% CI) between parameters and seasons, Electrical Conductivity, Total Phosphorus Total Dissolved Solids and Total Suspended Solids for the lower basin of the Usumacinta River, Tabasco, Mexico.

CONCLUSIONS

Anthropogenic activities and characteristics such as type and use of soil in areas near the sampling sites did not significantly influence temperature, pH, EC, TDS, TSS, total phosphorus and N-nitrates during the study period. However, the presence of frequent and high intensity rainfall during the rainy season, together with the planting and fertilization activities in rainfed crops, shows a tendency for an increase in the concentrations of TSS and total phosphorus. The decrease in river flow during the dry season causes an increase in surface water temperature and low availability of water for the dilution of solids, which marks an increase in the concentrations of TDS and electrical conductivity. It was determined that during the study period, the maximum values of TDS, TSS, total phosphorus and total coliforms exceed the permissible limits of NOM-001-SEMARNAT-1996 and the Ecological Criteria of Water Quality. Likewise, the presence of *Escherichia coli* was recorded in all the sites and during all the sampling months. This represents a contamination risk for the users of river water in these localities. In this study, diffuse contamination may be one of the factors that increase the concentrations of phosphorus and dissolved solids, particularly at the R1 Boca del Cerro and R2 Puente Chablé sites.

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AGRO PRODUCTIVIDAD
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Physiological response of chickpea (*Cicer arietinum* L.) in symbiosis with arbuscular mycorrhizal fungi under salinity conditions

Meriño-Hernández, Yanitza[1]; Garatuza-Payán Jaime[2]; Argentel-Martínez, Leandris[3]; Peñuelas-Rubio Ofelda[3*]; García-Urías, Julio C.[3]; Dell Amico-Rodríguez, José M.[4]; Rodríguez-Yon, Yaquelín[4]

[1] Universidad de Granma, Carretera a Manzanillo, Km 17 ½ Peralejo, Bayamo, Granma, Cuba. C.P. 85100.
[2] Instituto Tecnológico de Sonora. Departamento de Ciencias del Agua. Calle 5 de Febrero 818 Sur, Colonia Centro. Cd. Obregón, Sonora, México. C.P. 85000.
[3] Tecnológico Nacional de México / Instituto Tecnológico del Valle del Yaqui, Avenida Tecnológico s/n, Block 611, Bácum, Sonora, México. C. P. 85276.
[4] Instituto Nacional de Ciencias Agrícolas, Tapaste Km 3½, San José de las Lajas, Mayabeque, Cuba. C.P. 32700.
* Correspondence: openuelas.rubio@itvy.edu.mx

ABSTRACT

Objective: Evaluate the effect of salinity due to NaCl on physiological variables of chickpea plants using native strains of arbuscular mycorrhizal fungi (AMF) *Glomus cubensis* and *Rhizoglomus irregulare* during the pre-flowering stage.

Design/methodology/approach: The research was carried out under controlled weather conditions using the chickpea variety N-29 as an experimental model. The treatments consisted on the combination of four salinity levels: 0, 25, 50 and 75 mM NaCl (variation source A) and AMF (variation source B) in three levels. In total there were 12 treatments with six repetitions, which were distributed in a completely randomized experimental design. The evaluated variables were number of green and dry leaves, dry biomass per organ (leaves, root and stem), net assimilation rate (NAR), relative growth rate (RGR) and leaf area ratio (LAR).

Results: The green leaves, the NAR and the dry biomass from roots and leaves, were the variables with the greatest response in the 50 mM NaCl+*R. irregulare* treatment, with an average increase of 15% with respect to the rest of the treatments.

Limitations/implications: A decrease on the evaluated variables was observed due to the salinity effect; however, chickpea plants subjected to NaCl 50 mM inoculated with *R. irregulare* were less affected by salt stress.

Findings/conclusions. The *R. irregulare* strain was found to contribute more than the *G. cubensis* to the mitigation of the adverse effects from the salinity factor.

Keywords: *Glomeromycota*, plant development, abiotic stress, NaCl.

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INTRODUCTION

Salinity is one of the fundamental problems facing agriculture on approximately 20% of the world's irrigated lands, and salinity in these areas is increasing due to other factors such as low precipitation and bad cultural practices, with the expectation of an increase of up to 50% by the year 2050 (Shrivastava and Kumar, 2015). Saline stress seriously affects cellular

Image by Alex Dante at Pixabay

homeostasis causing ionic toxicity which reduces the development and productivity of cultivable species (Joshi *et al.*, 2017).

To reduce the effects caused by salinization on the crop's productivity, it is necessary to use tools and mechanisms that provide tolerance to this factor (Cardona *et al.*, 2017). Arbuscular mycorrhizal fungi (AMF) can be found in most soils and can form a symbiotic association with the majority of vascular plants, which allows the improvement of water and nutrients absorption, while conferring greater tolerance to different types of stress, both biotic and abiotic (Smith and Read, 2008). During the past few years, it has been shown that the successful inoculation of plants of agricultural interest with AMF promotes growth and improves plant performance, as a result of an improved absorption of nutrients (Cauich-Cauich *et al.*, 2022).

AMF are important bioenhancers for saline soils, since arbuscular mycorrhizal colonization mitigates the damage caused by soil salinization and improves the plant's growth, nutrition, and vigor (Evelin *et al.*, 2014). The plant-AMF symbiosis varies in form and efficacy depending on the plant, species and even on the genotype of the same species (Cobb *et al.*, 2016). In plants that thrive in saline conditions, mycorrhizal colonization can also improve root hydraulic conductivity at low water potential, stimulate and change the root system morphology and increase stomatal conductance (Latef *et al.*, 2016).

Chickpea (*Cicer arietinum* L.) is a legume of commercial importance and is consumed due to its nutritional properties, representing a great option manly due to its high protein content (Aguilar-Raymundo and Velez-Ruiz, 2013). 14.2 million Mg are produced annually around the world, in 14.8 million hectares, with a productivity of 0.96 Mg ha^{-1} (FAOSTAT, 2014), India being the first country in terms of production and productivity.

Chickpea is particularly sensitive to salinity (Flower *et al.*, 2010); efforts to find the difference in salinity tolerance have been undertaken by several authors (Hirich *et al.*, 2014).

The identification of chickpea crops that form an efficient symbiosis with mycorrhizal fungi and other endophytes could improve the physiological, biochemical an agronomic response of this species, when established in saline ecosystems (Bazghaleh *et al.*, 2018). There are currently few research on the AMF and chickpea plants grown in soil salinity conditions, for this reason, the current research's objective is to evaluate the crop's physiological response when established in NaCl based salinity conditions using two strains of AMF, as possible mitigators of the adverse effect of salt stress, in the chickpea 'N-29' crop, under experimental conditions.

MATERIALS AND METHODS

Location and experimental conditions

The experiment was established in a growth chamber with artificial lightning, with a photoperiod of 12 h, a daytime temperature of 26 °C and a nighttime temperature of 18 °C, until the flowering phenophase. The seeds used were treated previously with a 1% sodium hypochlorite solution, exposed for 10 min and then washed five times with sterile distilled water. Next, they were pre-germinated in sterile petri dishes at room temperature for 4 d, until the radicle and plumule emission. Subsequently, they were transplanted into plastic containers with 700 g of Fluvisol soil (Hernández *et al.*, 2015). The AMF inoculate was applied during the transplant.

A completely randomized design with factorial arrangement was used where two factors were evaluated: the salinity factor (A), with four levels corresponding to different concentrations of NaCl: 0, 25, 50 and 75 mM and a second factor (B) which was the soil inoculation with AMF strains in the next three levels: without AMF, *Glomus cubense* (Rodríguez *et al.*, 2011) and *Rhizoglomus irregulare* (Sieverding *et al.*, 2014), at a rate of 2 g per plant (45-50 spores g^{-1}). 12 treatments with 10 biological replicates were evaluated. The different saline solutions were applied twice per week, at a rate of 50 mL per plant. In the treatments that did not contain salinity, the same amount of water was applied and with the same frequency.

Evaluated variables

The number of green and dry leaves were evaluated, counting the existing quantity; and the leaf area using the Photoshop CS6 software. Likewise, the growth rate: net assimilation rate (NAR) through the relationship between values of the leaf area and the dry weight of the plant, expressed in g dm^{-2} d^{-1}; the relative growth rate (RGR) by using the formula:

$$RGR = \left[\left(final\ height - initial\ height \right) / number\ of\ elapsed\ days \right]$$

and the leaf area ratio (LAR) according to the methodology described by Beadle (1993). The dry biomass per organ (root, stem, and leaves) was determined according to the methodology described by Cardona *et al.* (2016).

Statistical analysis

For data processing, accomplishment of the theorical assumptions of homogeneity of variance was verified. Subsequently, a double classification analysis of variance was carried out, based on a linear model of fixed effects (Fisher, 1937) and in cases where there were significant differences between the averages, these were compared by using the Tukey test ($p \leq 0.05$) (Tukey, 1960). The determined indicators were standard error in the mean of treatments, coefficient of variation and unadjusted coefficient of determination for each variation source and for the interaction. The professional statistical package STATISTICA version 8.0 for Windows was used for all these analyses (StatSoft, 2008).

RESULTS AND DISCUSSION

In the green leaf's variable, there were significant differences between the established treatments ($p=0.0177$) (Figure 1). The greatest contribution to the total variability found in this variable was attributed to the salinity factor, contributing 52% ($R^2=0.52$), however, AMF contributed 36% ($R^2=0.36$), with a significant interaction between both, salinity factor and AMF, which contributed 10% ($R^2=0.1$).

In the dry leaves variable (Figure 1), salinity contributed 56% ($R^2=0.56$) of the total variability of the treatments; AMF contributed 27% ($R^2=0.27$) and there was also a highly significant interaction, contributing 16% ($R^2=0.16$). The obtained result demonstrates the

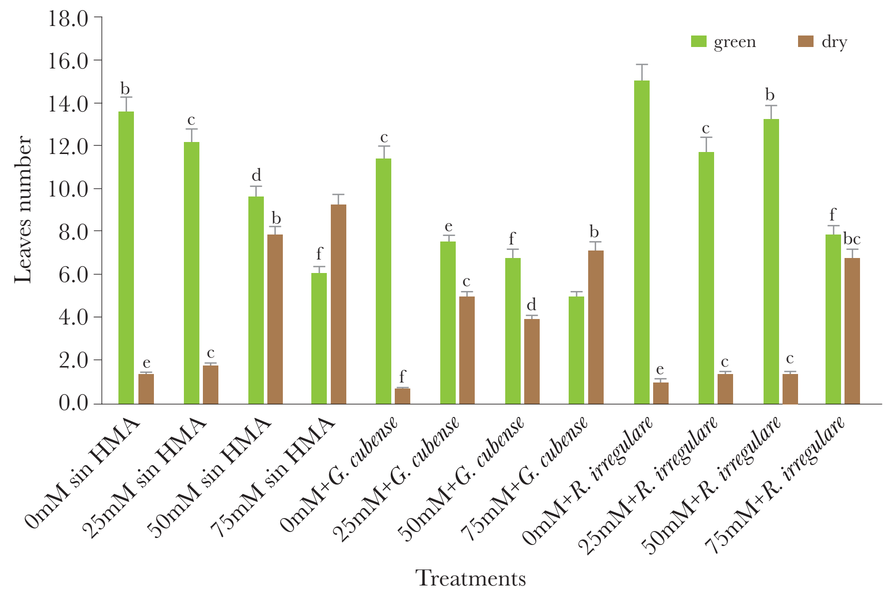

Figure 1. Number of green and dry leaves from the chickpea crop exposed to different NaCl levels inoculated with AMF. The vertical bars indicate the standard error of the means.

existence of a considerable sensitivity of the variety used in salinity, which can be degraded with the use of R. irregulare in agricultural practices in soils affected by this stressful condition. Cardona *et al*. (2016) indicated the use of these variables as precise indicators of salinity tolerance in the chickpea crops.

The number of green leaves decreased in the rest of the treatments, and the lowest values were registered on those plants that were under the NaCl effect without inoculation. Salinity is one of the main factors of environmental stress that directly affects the crop's development and productivity (Cardona *et al*., 2017). Multiple studies have been aimed to finding alternatives that can mitigate the stress effects on the plants, including the use of AMF. Harris-Valle *et al*. (2011) suggest that native AMF corporations allow a better growth and water balance for the plants, by increasing leaf water by more than 82% under salinity conditions of 40 mM NaCl. Nakmee *et al*. (2016), found an increase of 23% in the biomass and 29% in the dry weight of sorghum grain (*Sorghum bicolor* L.) compared to the regulation without AMF inoculation.

Meriño *et al*. (2018) evaluated these indicators in different chickpea crops under salt stress effect and found a significant reduction in the number of green leaves at 50 mM NaCl. Elhindi *et al*. (2017), when evaluating the mycorrhizal effect under different levels of induced salt, like the established in the current essay, found significant reductions in the number of leaves, number of branches and dry matter from the organs in both mycorrhized plants and non-mycorrhized plants, which demonstrates the susceptibility of this species to the aforementioned abiotic factor.

The amount of dry biomass in the three measured organs (root, stem and leaves) also showed a significant decrease as NaCl concentration increased; however, when *R. irregulare* was applied, the effects decreased in more than 10% on the treatments inoculated with this strain, obtaining a greater biomass from leaves and roots. The stem biomass was not significantly affected in the salinity treatments, but it did increase with the AMF inoculation (Table 1). In each evaluated organ, the total variability found (CV) was explained, in at least 97% (R^2), by the treatment's effects, which shows that with the linear model of fixed effects, used for statistical processing, was feasible to establish the differences between treatments.

These significant differences in the biomass production in the different salinity levels in both mycorrhized plants and non-mycorrhized plants could be related to the fact that the plants that were at the 75 mM saline level, did not overcome the possible nutritional interference generated in the absorbent complex of the soil by the Na^+ and Cl^- cations with respect to the essential nutrients required by the plants, or due to ionic toxicity caused by the low selectivity of the membranes or the non-activation of physiological, biochemical and/or molecular mechanisms of tolerances to the salinity conditions (Argentel *et al.*, 2016).

However, the plants that were in symbiosis with *R. irregulare* at a 50 mM NaCl concentration, presented a greater biomass. The current result shows the importance of the use of *R. irregulare* as a mitigator of the effects of salt stress in the variety used as an experimental model. In this regard, Cardona *et al.* (2017) reported similar results to the ones found in the current research, in Castilla Blackberry (*Rubus glaucus* Benth) in symbiosis with AMF exposed to different salt concentrations. Datta and Kulkarni (2014) found similar results to the ones reported on the current research, but on *Acaica arábica* crops under salt stress in mycorrhizal symbiosis and under similar conditions to the ones presented here.

Table 1. Dry biomass by organs (root, stems and leaves) from the chickpea crops inoculated with two AMF strains subjected to NaCl stress.

Treatments NaCl (mM), AMF	Dry biomass (g) (x±ES)			
	Leaves	Stem	Root	Total
0mM, without AMF	0.68±0.02 a	0.17±0.01 a	0.24±0.02 a	1.08±0.02 a
25mM, without AMF	0.61±0.05 ab	0.12±0.01 bc	0.19±0.01 bc	0.92±0.06 abc
50mM, without AMF	0.52±0.03 bcd	0.11±0.00 bcd	0.15±0.01 d	0.78±0.03 cd
75mM without AMF	0.43±0.09 cd	0.08±0.01 e	0.15±0.01 d	0.65±0.10 de
0mM+*G. cubense*	0.65±0.01 ab	0.11±0.01 bcd	0.18±0.02 bcd	0.94±0.02 ab
25mM+*G. cubense*	0.61±0.02 ab	0.09±0.00 cd	0.17±0.01 cd	0.87±0.03 bc
50mM+*G. cubense*	0.56±0.04 abc	0.12±0.01 bc	0.15±0.01 d	0.82±0.04 bc
75mM+*G. cubense*	0.51±0.02 bcd	0.12±0.01 bc	0.19±0.02 bcd	0.81±0.04 bc
0mM+*R. irregulare*	0.69±0.07 a	0.14±0.01 ab	0.22±0.01 ab	1.04±0.09 a
25mM+*R. irregulare*	0.59±0.02 ab	0.14±0.01 ab	0.22±0.02 ab	0.95±0.04 ab
50mM+*R. irregulare*	0.67±0.03 a	0.14±0.01 ab	0.24±0.01 a	1.05±0.03 a
75mM+*R. irregulare*	0.39±0.06 d	0.09±0.01 cd	0.15±0.01 d	0.62±0.06 e
CV	3.5	2.9	4.1	5.1
R^2	0.98	0.99	0.99	0.97

Different letters in the same column indicate significant differences, by Tukey ($p \leq 0.05$). ESx: Standard error of the mean.

The initial leaf area did not show significant differences, perhaps due to the little or no absorption of toxic cations and/or anions. However, the negative effect of salt stress at the end of the experiment was greater, with a variation percentage greater than 18%.

At the end of the experiment, a significant increase in the leaf area was found, against salinity, with a greater response when the plants where in symbiosis with *R. irregulare* at 50 mM NaCl. The previous results show that the inoculation with *R. irregulare* allowed the plants to acquire an adaptation to saline stress as the experiment developed.

These results agree with the those obtained by Abdul (2011) in the bean crops, which showed a 25% decrease in the leaf area after 10 d of applying 60 mM NaCl. Likewise, Abdolahpour and Lotfi (2014), observed a significant loss, greater than 40% of the leaf area with 75 mM NaCl in chickpea.

The net assimilation rate (NAR) decreased significantly in treatments where the highest concentration of NaCl (75 mM) was applied, even when AMF was inoculated. The percentage of variability found in this indicator was 14% (Table 2).

The fact that the NAR remained similar in the 0 mM NaCl and 50 mM+*R. irregulare* treatments demonstrates the contribution of this strain to the mitigation of the adverse effects of NaCl on the chickpea crop development.

The RGR and LAR decreased as NaCl concentration and stress exposure time increased. In both variables, the total variability found (CV) was explained in more than 97% (R^2) by the effect of the established treatments. The reduction on the treatments with 75 mM NaCl with and without AMF, were approximately 50% when compared to the treatments with 0 mM NaCl (Table 2). In return, plants subjected to concentrations of 50 mM with *R. irregulare* showed similar response to those of the controls.

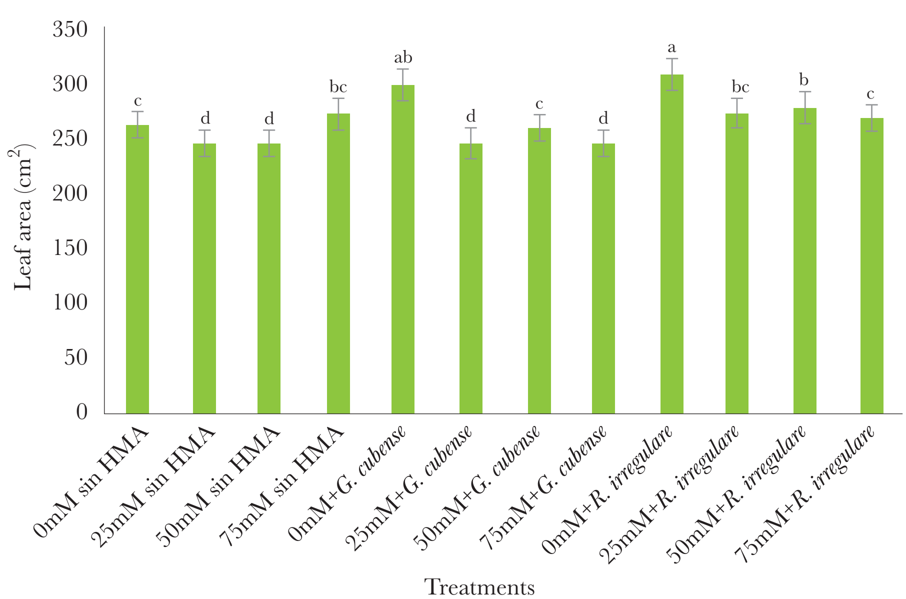

Figure 2. Chickpea plants leaf area in symbiosis with AMF exposed to different salt concentrations. (Different letters in the columns indicate significant differences, by Tukey ($P \leq 0.05$). Coefficient of variation: CV=19.3%).

Table 2. Growth and development indicators.

Treatments NaCl (mM), AMF	Development indicators (X±ESx)		
	RGR (cm day^{-1})	LAR (cm^2)	NAR (mg cm^{-2} dm^{-1})
0mM without AMF	4.99±0.18 a	134.00±2.21 bc	0.33±0.0014 a
25mM without AMF	4.26±0.48 abc	127.47±2.58 bc	0.26±0.0011 c
50mM without AMF	3.87±0.23 abc	125.46±2.40 c	0.26±0.0011 c
75mM without AMF	2.75±0.70 bc	125.36±4.39 c	0.18±0.001 d
0mM+*G. cubense*	4.91±0.40 a	142.03±3.15 abc	0.30±0.002 b
25mM+*G. cubense*	4.44±0.30 abc	131.22±1.52 bc	0.25±0.001 c
50mM+*G. cubense*	3.86±0.38 abc	129.75±2.43 bc	0.22±0.001 c
75mM+*G. cubense*	3.68±0.22 abc	127.26±4.78 bc	0.21±0.001 cd
0mM+*R. irregulare*	5,06±0.40 a	158.64±4.75 a	0.32±0.0013 a
25mM+*R. irregulare*	4.59±0.43 ab	148.20±4.04 ab	0.27±0.0011 bc
50mM+*R. irregulare*	4.87±0.04 a	145.93±2.82 abc	0.32±0.0013 a
75mM+*R. irregulare*	2.65±0.49 c	126.94±2.21 bc	0.15±0.0006 e
CV	7.21	5.3	14.5
R^2	0.99	0.98	0.72

Different letters in the same column indicate significant differences, by Tukey ($p \leq 0.05$). ESx: standard error of the mean. RGR: relative growth rate; LAR: leaf area relation; NAR: net assimilation rate.

A similar trend can be observed in the LAR, where there was a significant reduction in the treatments with NaCl without inoculation and those inoculated with *G. cubense*, without significant differences between them. When the plants grew in symbiosis with *R. irregulare*, the leaf surface increased with respect to the breathing/respiratory mass (total biomass), not being so in those treatments subjected to 75 mM NaCl in which the greatest reductions are shown in this indicator.

Balliu *et al*. (2015), found that the relative growth rate decreased less when the plants where in symbiosis with the mycorrhizae under saline conditions, being the non-mycorrhizal plants the most affected. In experiments carried out by Miranda *et al*. (2011) it was shown that the leaf area ratio and the photosynthetic rate in the mycorrhized plants increased with respect to non-mycorrhized plants under saline conditions.

The research carried out revealed the variation in the response of the chickpea 'N-29' variety to salinity and the feasibility of using AMF to mitigate issues related to soil salinity. This result will contribute to increase the use of saline soils and food self-sufficiency in regions affected by this type of stress, mainly in areas where other species fail to reach their productive genetic potential and food production needs are more than necessary, essential.

CONCLUSIONS

In the chickpea 'N-29' variety, there was a reduction in the development variables as the saline concentration increased; however, these effects decreased with the use of *R. irregulare*.

Given the salinity conditions dictated, the variables with a greater response to stress were number of green leaves, the dry biomass of roots and leaves, and the net assimilation rate (NAR). These variables can be used as reference indicators for salinity tolerance in chickpea when arbuscular mycorrhizal fungi are used.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Positive impact of business efficiency sub-factors and variables on the competitiveness of the lemon agro-industry in Colima, Mexico

Magaña-Sánchez, Pablo A.*

Universidad de Colima, Campus El Naranjo, Carretera Manzanillo-Cihuatlán km 20, Ejido El Naranjo, Manzanillo, Colima, México, C. P. 28864.

* Correspondence: pablo_magana@ucol.mx

ABSTRACT

Objective: To establish which business efficiency sub-factors and variables have a positive impact on the competitiveness of the lemon agro-industry in Colima.

Design/Methodology/Approach: In this mixed research, we used a questionnaire with 33 questions with a scale of 1 to 6. We applied the questionnaire in the field to nine managers and established its validity and reliability using Cronbach's alpha (0.91). We used variable standardization and the arithmetic mean as statistical tools. With this technique, we integrated 33 variables in one index. We considered different means for each of the companies and used the software SPSS version 22.

Results: Labor market and management practices were the sub-factors with the highest number of variables that have a positive impact on the competitiveness of the lemon agro-industry in Colima.

Study Limitations/Implications: The study limitations included the lack of time and availability of the interviewees, as well as the expenses in which we incurred traveling to their companies.

Findings/Conclusions: The variables with the highest positive impact on the competitiveness of the lemon agro-industry in Colima are the following: customer satisfaction is very important; security and hygiene-related issues are adequately addressed; social responsibility is high; and attracting and retaining talents is a priority. We concluded that business owners within this agro-industry seek to create good work teams, offer training opportunities, provide good security and hygiene conditions, and have an adequate financial control.

Key words: Competitiveness, Lemon agro-industry, Questionnaire, Labor market, Customer satisfaction.

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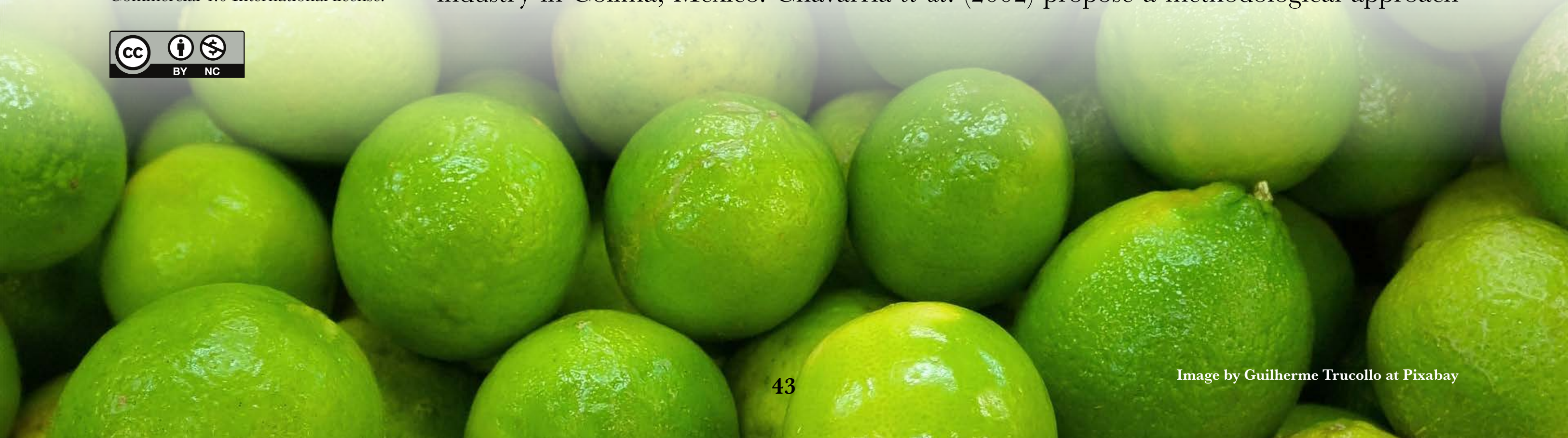
INTRODUCTION

This study is based on state-of-the-art business efficiency and competitiveness. The term "business efficiency" implies the optimization of all activities that companies carry out for their own benefit and that of their partners. In other words, a company is efficient in business when it makes the most of its resources and adapts to change in order to be competitive. This leads us to our second topic, *i.e.*, competitiveness. We must discuss the state-of-the-art regarding this topic in the context of the companies we are studying within the lemon agro-industry in Colima, Mexico. Chavarría *et al.* (2002) propose a methodological approach



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that integrates the main components of agricultural competitiveness and links them in a fitting manner with the process of sustainable development of agriculture. This theoretical approach is based on two units: 1) the agri-food chain and its corresponding links and agents; and 2) the territorial units where the different productive agents are located. The latter unit is based on certain elements of the location theory of development. In addition, it lays the foundations for the inclusion of a number of infrastructure components that support production and the availability of natural resources. In this context, agricultural competitiveness can be defined as a comparative concept based on the dynamic capacity of an agri-food chain spatially located to maintain, expand, and constantly and steadily improve its participation in both domestic and international markets. All this is achieved through the production, distribution, and sale of goods and services at the required time, place, and form, with the ultimate goal of benefiting society (Chavarría *et al.*, 2002). The competitiveness of the agri-food sector is its capacity to place its produced goods in the market under fair competition conditions, achieving the welfare of the population (García, 1995). The competitiveness of agri-food chains has a close relation to sustainable development. Regional competitiveness can be defined as the management of resources and capacities to steadily increase business productivity and the welfare of the regional population (CEPAL, 2010).

Since the late 20^{th} century, globalization has prevailed as one of the main traits of international economy; it is characterized by a convergence towards more open and competitive markets (Rojas and Sepúlveda, 1999). The concept of agricultural competitiveness must be understood within the context of sustainable development, which comprises four dimensions: social, political-institutional, environmental, and economic (Rojas and Sepúlveda, 1999). The objectives of these dimensions are equity, governance, sustainability, and competitiveness, respectively. The Asia-Pacific Cooperation Forum's (APCF) understanding of competitiveness acknowledges that the new world economy is characterized by market globalization and by a new era of information, knowledge, and ongoing, fast, and uncertain change. Therefore, this perspective complements the approach based on productivity and economic policy by grouping it in four dimensions: business environment, innovation system, human resources development, and a high-capacity information and telecommunications infrastructure. This leads to a broader scheme of competitiveness (Soto-Ramírez, 2005) with different national and international approaches. In the case of Mexico, real competitiveness is based on productivity, skilled labor, logistics, tariff structure, innovation, technological development, and democracy. According to the IMD - World Competitiveness Center (WCC), there is an international competitiveness of nations, an international sectoral competitiveness, and an international company competitiveness (Garelli, 2004). The competitiveness of nations seeks to create and maintain an environment that sustains company competitiveness (Krugman, 1994). Competitiveness is a country's ability to create, produce, distribute, and/or service products in international trade, simultaneously earning higher returns on their resources (Scott and Lodge, 1985; Otero *et al.*, 2006).

The definition of the concept of competitiveness takes into account three analytical levels: micro, meso, and macro. Coincidences were found in the micro and meso levels; however, there is some disagreement regarding the macro level. In this sense, reference is made to economist Paul Krugman, who criticizes the use of the concept of national competitiveness (Lombana and Rozas-Gutiérrez, 2009; García-Ochoa *et al.*, 2017). In order to leave this debate behind, the authors suggest using the term "competitive environment of a nation" (within each country), instead of referring to "competitiveness of nations" (among countries). In 1989, the Economic Commission for Latin America and the Caribbean (ECLAC) and the United Nations Industrial Development Organization (UNIDO) pointed out that competitiveness is the capacity to sustain and increase participation in international markets while simultaneously improving the living standards of the population (Zamora *et al.*, 2013). Meanwhile, in its 1997 report, the Competitiveness Advisory Group states that the competitiveness of a nation or region can be observed in its ability to develop several factors that are essential for long-term economic growth, such as productivity, efficiency, specialization, or profitability (Zamora *et al.*, 2013). Competitiveness is the capacity to combine quality, price, design, and delivery time in order to meet the customer's needs and eventually make the company a reliable link of the productive chain that includes other companies that offer similar products (Inmujeres, 2009; Saavedra-García, 2020).

Porter (1990) argues that developing a widely accepted theory to explain a nation's competitiveness is more relevant than adopting any of the concepts proposed for the term. The competitiveness of nations has been linked to variables such as type of currency, type of interest, and budget deficit. According to Krugman (1990), an analysis of a nation's competitiveness should consider the different determinants of the population's living standards (*e.g.*, growth, employment, and wage distribution). Porter (1987) believes that a nation's competitiveness could be defined as the degree to which, under open market conditions, the nation can produce goods and services that satisfy the test of international markets, while at the same time it maintains and expands its citizens' long-term real income. The Organization for Economic Co-operation and Development defines competitiveness as the ability of companies, industries, regions, and countries to generate income, as well as high employment levels, in a sustainable manner, while being exposed to international competition (OCDE, 1997).

Porter (1990) indicates that a country's competitiveness depends on the ability of its industries to innovate and improve. One indicator of a country's competitiveness is the performance of its exports in international markets. According to Amaral (1992), competitiveness is a socioeconomic organization's ability to conquer, maintain, or expand its market participation in a profitable way that allows its growth. As stated by Ruiz (1995), the basic competitiveness level, according to the systemic approach, comprises the companies and the factors that affect their capacity to compete. Competitiveness is the ability to push back the limits of external restrictions: the higher a country's capacity to compete in its own or in foreign markets, the higher the economy's capacity to grow unhindered by external deficit (Bejarano-Ávila, 1998). Calva (2007) states that competitiveness is a complex phenomenon in which multiple interrelated factors intervene; for this reason, it is considered systemic. According to Ramos (2001), a country's competitiveness can be currently measured and quantified based on the World Economic Forum's (WEF) Global Competitiveness Report and the International Institute for Management Development's

(IMD) World Competitiveness Yearbook. Both measurement tools have used similar competitiveness factors to analyze the economies of the countries included in their reports and arrange them in order of importance. The IMD classifies data based on four main competitiveness factors: economic performance, government efficiency, business efficiency, and infrastructure. Each of these factors has five sub-factors. In the case of business efficiency, the subject matter of this study, the five sub-factors are productivity and efficiency, labor market, finance, management practices, and attitudes and values. The study's main purpose is to establish which business efficiency sub-factors and variables have a positive impact on the competitiveness of the lemon agro-industry in Colima. The aim of this study was to obtain information that can be useful for the managers of the studied companies. It will serve as reference for researchers who want to expand their knowledge in this area.

MATERIALS AND METHODS

This research is mixed, *i.e.*, it is both qualitative and quantitative. As a data collection tool, we used a questionnaire with 33 questions with a 1-6 scale and applied it to nine companies. Cronbach's alpha (0.91) was used to establish the questionnaire's validity and reliability. The questions were designed based on the business efficiency factor and its five corresponding sub-factors. The interviewees were nine managers of companies within the lemon agro-industry in Colima. Afterwards, we used variable standardization and the arithmetic mean to determine the sub-factors and variables that influence the competitiveness of the lemon agro-industry in Colima. Both statistical tools were used to integrate the 33 variables into a single index. Different means were considered for each of the companies using the SPSS software. Subsequently, these means were standardized, and the resulting mean value was 50%. In other words, the median is 50%; therefore, results that show values over 50% are above the mean in terms of competitiveness. This is how we determined the sub-factors and variables that have a positive impact on the competitiveness of the studied companies.

RESULTS AND DISCUSSION

In total, the five business-efficiency sub-factors included 23 variables that have a positive impact on the competitiveness of the lemon agro-industry in Colima, Mexico.

According to one variable of the productivity and efficiency sub-factor, the knowledge and use of international standards favors companies within the lemon agro-industry, providing them the possibility of exporting their products. Within the labor market sub-factor, the following variables had a positive impact on competitiveness: labor costs are high; labor relations usually tend towards cooperation and teamwork; training of management and operative staff is a high priority activity. The local, state, and regional labor market offers qualified staff, able to develop the companies' economy; the demand of staff with professional and specialized knowledge is therefore widely met on a state and national level. Likewise, attracting and retaining talent is an important activity in the lemon agro-industry in Colima; operative employees have a well-developed career plan and the companies try to retain them. Senior managers have a wide domestic and international business experience.

The state and regional markets offer a pool of candidates with at least undergraduate studies who can work in management positions (*e.g.*, supervisor, head of department, or manager) and who will be able to ensure the companies' financial development.

Within the finance sub-sector, the impact variables were the minimal requirements established by banking institutions to grant credits to the studied companies helps them to achieve financial development; self-financing by means of cash flow is also relevant.

The impact variables within the management practices sub-factor were the following: the studied firms are highly adaptable to market changes; ethical practices are implemented in all their activities; the board of directors has an efficient and effective management control; auditing and accounting practices are adequately followed based on the accounting principles established by regulations. Customer satisfaction is very important for these companies; managers show an entrepreneurial spirit; the companies have high levels of social responsibility; security and hygiene issues are addressed in a strict and adequate manner, following the rules set by the Ministry of Health.

Finally, the attitudes and values sub-factor included the following impact variables: flexibility and adaptability to new challenges are high; the organizational values system practiced by the operative and the management staff fosters competitiveness.

CONCLUSIONS

We conclude that, out of all the business efficiency sub-factors, labor market and management practices have the highest number of variables with a positive impact on the competitiveness of the lemon agro-industry in Colima. Business owners attach great importance to their internal and external customers. They seek to create cooperative work teams among their internal customers (*i.e.*, their employees) and retain them for as long as possible. Therefore, it is of the utmost importance for them to train their staff and provide them with good security and hygiene conditions to carry out their daily activities. Of similar importance, employees that work in these companies —whether they are part of the management or the operative staff— are trained and available in the region, and management staff has enough domestic and international business experience.

Regarding their external customers, business owners seek to fully apply marketing tools (*i.e.*, to meet all their customers' needs and wishes and to ease their fears). These companies easily adapt to market changes, since their staff has the right attitudes and abilities to adequately fulfil their duties, carry out their activities in an ethical manner, has an adequate financial control, and is socially responsible. Our findings help business owners of the lemon agro-industry in Colima, Mexico in their decision-making and are also useful to develop new lines of research about companies within the lemon agro-industry, both in Mexico and elsewhere.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Characterization of producers who use wastewater for irrigation of vegetables and forages

Neri-Ramírez, Efraín[1]; Rubiños Panta, Juan Enrique[2*]; Delgado Martínez, Rafael[1]

[1] Universidad Autónoma de Tamaulipas, Centro Universitario Victoria, Blvd. Adolfo López Mateos s/n, Col. Centro, Ciudad Victoria, Tamaulipas, México, C. P. 87134.

[2] Colegio de Postgraduados, Programa de Hidrociencias, Carretera México-Texcoco km 36.5, Montecillo, Estado de México, México, C. P. 56230.

* Correspondence: jerpkike@colpos.mx

ABSTRACT

Objective: To characterize the production systems of the users of Chilhuacán canal waters located in Atlixco, Puebla and to identify the role they play in the persistence of wastewater use in agriculture.

Design/Methodology/Approach: A characterization of regional producers was conducted applying a questionnaire and making field visits to the producers' plots.

Results: There are seven types of producers who specialize in different types of crops (forages and vegetables); they also use agricultural areas of different sizes (<3ha, ≤6ha and >6 ha).

Limitations/Implications: Wastewater use in agriculture is a recurring phenomenon in the world (including Mexico). As a result of the low water availability, wastewater is used to irrigate crops for human consumption.

Findings/Conclusions: The awareness of the producers about the impact of this practice is low and they have no interest in the harmful polluting effects on their plots and their health.

Keywords: Health, pollution, environmental deterioration, typology of producers.

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INTRODUCTION

Nowadays, the Mexican field faces serious problems, but doubtlessly, environmental deterioration is one of the most important. A significant component of this phenomenon is the pollution of rivers, springs, and even the water extracted from deep wells, resulting in serious human health problems —both directly or through the food chain. The state of Puebla plays a key role in the domestic production of vegetables, flowers, and forage. The municipality of Atlixco is known within the state by the widespread sowing of vegetables (coriander, radish, onion, lettuce, and zucchini), flowers (gladiolus, cockscomb, chrysanthemum, and common baby's breath), and forage (alfalfa, corn, and oats). The municipality of Atlixco has a flat soil broken by the Popocatépetl foothills.

Image by Paul Merino at Pixabay

According to the 2010 Population Census data, Atlixco has a population of 127,062 habitants (hab), with a population density of 432.02 hab km^{-2} (INEGI, 2010). The Chilhuacán canal is in this municipality. Its waters come from a deviation of the Nexapa river into which untreated industrial and urban wastewater is discharged; this highly-polluted river also drags a large amount of garbage throughout its riverbed. Despite the pollution of the canal and the increasing pollution rates, these waters have been used for years in agricultural production. Therefore, their use for these purposes has been questioned, since according to NOM-001-SEMARNAT-1996 these waters are categorized as "waters whose agricultural use is subject to certain conditions": their use is restricted for horticultural crops and grains, although it has been allowed for forage and ornamentals. However, due to traditional use conditions and to the lack of alternative water supplies for agriculture in the region, farmers seem highly unwilling to stop using wastewaters and, consequently, continue with this irrigation practice. To analyze the characteristics of those producers, producer groups with similar features and practices (typologies) were identified (Malagón and Prager, 2001). In addition, their economic model was identified according to their resources, level of technology, and the strengths and limitations of the area where they work (FAO/USAC, 1995). The characterization goal is to establish producer groups based on qualitative criteria (similarity), by means of which clear differences can be established between them, disregarding secondary heterogeneities (Apollin and Eberhart, 1999). The elaboration of typologies seeks to simplify diversity among the producers in the same region, by identifying production system groups (types) with similar potentialities and restrictions against one or several chosen elements (Amador *et al.*, 1995). The purpose of this work was to characterize the production systems of the users of Chilhuacán canal waters and to identify the role they play in the persistence of wastewater use in agriculture.

MATERIALS AND METHODS

The study area includes the irrigation zone of the Chilhuacán canal, located to the east of the municipality of Atlixco, encompassing the communities of Santa Ana Yancuiltlalpan, San Félix Almazán, Nexatengo, and La Ciénega. The canal is 4.5 km long and is used to irrigate 680 ha. The number of users per irrigation unit is showed in Table 1.

Table 1. Irrigation units of the Chilhuacán canal and number of users.

Watering units	Number of users
Ejido San Félix Almazán	164
Ejido Santa Ana Yancuitlalpan	86
Ejido Revolución	139
Ejido Flores Magón	104
Ejido Xonacayucan	24
Ejido Loma Larga	24
Pequeña propiedad Maurer	3
Pequeña propiedad Galeazzi	3

Source: User census of the irrigation module 07, Nexapeños del Norte A. C.

The questionnaire was designed based on Pérez's (1994) recommendations. To calculate the sample size, the total number of users of the canal was considered as the study population (547) and the statistical formula of maximum variance was used (Pérez, 1994; Triola, 2009), obtaining a sample size of 60 questionnaires. The following formula was applied to obtain the sample size:

$$\eta = \frac{N\left(\frac{Z_{\alpha}}{2}\right)^{2}(pq)}{N\,d^{2} + (Z_{\alpha})^{2}(pq)}$$

Where: η=sample size; N=population size; $\left(\frac{Z_{\alpha}}{2}\right)^{2}$=Reliability, with α=0.05 and pq=0.25 d=standard error, 10% (0.10).

The instrument consisted of three sections: 1) Characterization of production units which included questions about the general producer characteristics in the study area, taking into account factors such as age, level of education, number of family members, land area, crop census, and production costs as proposed by Tabares *et al.* (2000) and Rubio (2006); 2) awareness about the environmental problems caused by canal sewage; and 3) point of view of the users about the ongoing use of canal waters for agricultural purposes.

RESULTS AND DISCUSSION

The information collected was used to develop a database with 30 variables from which the most representative ones were selected, as proposed by Berdegué *et al.* (1990). First, the coefficient of variation (CV) of each variable was calculated and those with low discriminant power (<50% CV) were eliminated; subsequently, the association degree between them was determined. Consequently, only the 12 variables with the highest correlation and representativeness were selected (Table 2).

It was possible to differentiate the production limiting factors, the production unit objectives, type of production, technology, relationship with markets, labor, nature of costs, and rationality behind decision-making, as proposed by Valderrama and Mondragón (1999). Regarding the members of the family nucleus (Table 3), 16.4% consists of three or fewer people, 57.4% has from four to six members, and 26.2% is considered a large family (seven to 10 members). The second and third groups (83.6%) surpassed the national and state mean, which is three members per family (INEGI, 2010).

Most producers have a low educational level, although it is higher than in other regions of the state. Regarding land availability, the mean production unit has 3 ha, with a minimum of 0.5 ha and a maximum of 40 ha. The small producer group was made up of individuals with an average age of 55 years, with more than 35 years of experience growing vegetables, and with high school as their highest educational level. On average, the medium size producer is 40 years old, has undergraduate studies, and more than 20 years of experience in forage and vegetable production. The average age of large producers was 45 years old, with 25 years of experience, and an educational level

Table 2. Variables of forage and vegetable producers in Atlixco, Puebla, Mexico.

Variable	Description
A1	Number of family members
A2	Producer's age (years)
A3	Farmer's schooling (years)
A4	Producer's surface (hectares)
A5	Fodder area (hectares)
A6	Vegetable area (hectares)
A7	Income from forages ($ ha^{-1})
A8	Vegetable income ($ ha^{-1})
A9	Fodder yield (t ha^{-1})
A10	Yield of vegetables (t ha^{-1})
A11	Production degree of mechanization (high>80%, medium>60% and low <50%)
A12	Years of planting fodder crops and vegetables

Source: Table developed by the authors based on their own field work.

Table 3. Socioeconomic and productive characteristics of the producers.

Variable	Type of producer by farm size		
	Small	Medium	Extensive
Number of family members	2±3	4±6	7±10
Producer's age (years)	82±28	40	45
Farmer's schooling (years)	15±6	12	15
Producer's surface (hectares)	5.5±0.5	11.3	40
Fodder area (hectares)	47	11	40
Vegetable area (ha)	8	0.3	0
Income from forages ($ ha^{-1})	10000±8000	21000±18000	21000±18000
Income from vegetables ($ ha^{-1})	18000±15000	21500±19000	0
Fodder yield (t ha^{-1})	15±10	30±20	35±30
Yield of vegetables (t ha^{-1})	20±10	20±11	0
Degree of mechanization in production (high/medium/low)	50±30	70	100
Years sowing fodder and vegetables	40±25	20	25

Source: Table developed by the authors based on their own field work.

of postgraduate studies (Table 3). However, with regard to the age, educational level, and activity experience variables, Vilaboa and Díaz (2009) reported that older producers with low educational level and more experience —as was the case of small producers in the study area— have deep-rooted knowledge about production methods; consequently, they are considered reluctant to change. Meanwhile, producers with more activity experience and a higher educational level may find themselves in a transition process, with greater openness to change regarding both crops and the production methods. Furthermore, there were marked differences between producer groups regarding the productive variables of their plots. Producers with smaller area had low yields (10-20-ton hectares^{-1}), as a result of their cultural practices and labor. For example, the gravity method was the most used irrigation system; they used less machines for weeding and other agronomic practices. This situation restricted their knowledge about the new technologies they could have access to. Medium and large producers have a higher degree of mechanization and better technological packages that allow them to obtain better yields and a better price for their products (Table 3). Most of the operations belong to smallholders, since 96.72% has up to 5 hectares. Meanwhile, 1.64% can be considered as medium size (more than 5 and up to 11 ha) and another 1.64% of the operations are considered large (more than 15 hectares, without surpassing 40 hectares) (Table 4).

The prevailing landholding type is *ejido* (83.6%), followed by the *ejido*-small property combination (9.8%), small property (3.3%), *ejido*-rent combination (1.6%) and finally only rent (1.6%). Agricultural activities in the study area only have access to wastewater: there are no deep wells because it is a closed area. Therefore, there is a limited availability of water, which worsens the situation during the dry season. Consequently, many producers who work in areas greater than 3 ha buy hours of water from other users to supply the needs of their crops, an activity mentioned by Ramos *et al.* (2003). Regarding water cost, Chilhuacán canal users pay an annual fee, which is used to maintain the distribution system, since the cost per hectare of irrigation is around $100.00. Water distribution is shifted among the eight units per total volume of water carried through the canal (around 600 L s^{-1}); therefore, the users of each unit can irrigate at least twice a month. Most users irrigate their plots at night to avoid evaporation, as recommended by the Mexican Institute of Water Technology (IMTA, 2003). Users are aware that it is not recommended to use wastewater to irrigate vegetables, but they are unaware of the potential biological pollution of crops, as mentioned by Guzmán *et al.* (2007); nevertheless, they persist in this practice. Regarding cultivars (such as alfalfa and gladiolus), locally produced seeds are used in most cases. The most widely used agrochemicals in the region are the following: aldrin, dichloro-diphenyl-trichloroethane (DDT), methyl bromide, and parathion, which have been banned in many countries; however, they are still marketed due to poor regulation and control by local, regional, and national authorities (Drechsel *et al.*, 2002). The total area managed by the interviewees was 200 ha; the producers sow from one to three cycles per ha each agricultural year, with an average land operation index of 2.5 crops per year (equivalent to 500 ha). For this work, the three most representative crops were selected, grouping them into two types: forages (alfalfa) and vegetables (onion and coriander). According to Escobar (2003), determining production costs faces some difficulties, caused primarily by the diversity and origin of the inputs used, as well as the variation in the

Table 4. Classification of operations per area.

Classification	Surface area (hectare)	Frequency	(%)
Smallholders	0.5 a 5.5	59	96.72
Medium size	5.6 a 15	1	1.64
Large size	>15	1	1.64

Source: Table developed by the authors based on their own field work.

quantities which complicates its monetary variation. Surface-wise, alfalfa is the most important crop in the study area. Its average total cost of production is estimated to be $16,580.00 per ha. It is important to indicate that, on average, eight irrigations are applied per hectare for the establishment of this forage, considering that the approximate value of each of them is $100.00 per irrigation ha^{-1}. Consequently, using wastewater is more profitable than groundwater; however, from the environmental point of view this practice causes damage to soils and crops. Therefore, if we put together the prices of fertilization, growth, harvest, and forage packaging, the average value is $18,600.00 per ha. Meanwhile, the establishment and development process of onion is one of the most expensive, resulting in a development cost of $16,413.00 per hectare. If harvesting and transportation costs are added, the costs rise to $24,033.05 per hectare.

Finally, in the case of coriander, the establishment and development costs are remarkably like that of alfalfa (total cost: $18,550.55 per hectare). A great diversity of highly heterogeneous variables is involved in the structure and operation of farm units. Therefore, we recommend establishing classification mechanisms for their analysis; a cultivation typology reduces the existing diversity to a level that facilitates the analysis (Murmis, 1980; Berdequé *et al.*, 1990; Landín, 1990; Pérez, 1994; Coronel and Ortuño, 2005). A classification of cultivations was also established based on the following variables: 1) physical dimension and 2) diversity of the operation in terms of its specialization degree in agricultural production. Out of all the possible combinations of these variables, seven dominant types were identified (Table 5) and used as the basis of the analysis of the information collected.

The producers who showed the greatest awareness and interest in the levels of water pollution and its effects specialize in forage and ornamental flowers, crops whose irrigation with these waters is restricted; in this case, there is no health risk, since they are not intended for human consumption.

Table 5. Cultivation typology based on the selected variables.

Area	Number of users	Expertise	Number of users	Characterization
Smallholders (0-2.9 hectares)	34	Forager	22	Type 1. Small farm with dominant forage production.
		Ornamental flowers	4	Type 2. Small farm with dominant production of ornamentals.
		Horticulturist	8	Type 3. Small farm with dominant vegetable production.
Medium size (3-6 hectares)	25	Forager	22	Type 4. Medium farm with dominant production of fodder.
		Horticulturist	3	Type 5. Medium farm with dominant vegetable production.
Large size (>6 hectares)	2	Forager	1	Type 6. Large farm with dominant forage production.
		Horticulturist	1	Type 7. Large farm with dominant vegetable production.

Source: Table developed by the authors based on their own field work.

Furthermore, producers who have a lower awareness level and show little interest in the level of water pollution and its effects specialize in vegetable production. Overall, more than 50% of the users are aware of pollution and its effects; however, they are not interested in this problem, which is perhaps justified by the fact that wastewater is their only source of water. Some health problems (gastrointestinal and skin diseases) that could be caused by wastewater have been detected in most operations; however, producers do not realize that these diseases are the direct result of this practice. Most of the users are uninterested in this problem; this situation is a cause of concern, particularly regarding vegetable production units. Regarding the role that this practice plays for the family economy in the seven units, the agricultural activity is the highest source of income, and, in some cases, it is the only source of income for the family. In addition, the non-existent cost of water is an important aspect for the persistence of this practice in this region since producers only pay an annual fee for maintenance of the distribution system. Consequently, the irrigation cost is $100.00, making irrigation with surface waters from the Nexapa River relatively cheap, because they are polluted. In the future, producers expect to continue this practice, although they are aware of possible solutions that would allow them to stop using this type of water for agricultural purposes. They doubtlessly believe that the problems of plot pollution, as well as damage to the environment and human health, will increase in the future, in the case of operations type 6 and 7.

CONCLUSIONS

There are seven types of producers whose differences lay mainly in their cultivation size and crop specialization (particularly, typologies 1 and 4). Most of the producers are 50 years or older and in average their families have four members. Alfalfa is the predominant crop; vegetables and flowers hold the second and third places, respectively. On the one hand, the users do not care about the polluting effects on their plots and health caused using sewage. On the other hand, despite knowing that the water is polluted, they also cannot stop using it, since it is the only water, they have available. Despite the lack of interest about this problem and the possible solutions, a consensus was reached by most of the producers about the three ways in which this problem could be solved: 1) to establish a treatment plant, 2) to drill deep wells, and 3) to engage in a productive reconversion.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Behavior analysis of real wages in Mexico (1995-2018)

Pérez-Soto, Francisco[1*]; Figueroa-Hernández, Esther[2]; Varela-Ramírez, Mario A.[1]; Escamilla-García Pablo E.[3]; Jiménez-García, Martha[3]

[1] Universidad Autónoma Chapingo, Carr. México-Texcoco Km 38.5, Chapingo, Texcoco, Estado de México, México, C. P. 56230.
[2] Centro Universitario UAEM Texcoco, Universidad Autónoma del Estado de México, Av. Jardín Zumpango S/N, Fracc. El Tejocote, Texcoco, Estado de México, C.P. 56259.
[3] Instituto Politécnico Nacional.
* Correspondence: perezsotofco@gmail.com

ABSTRACT

Objective: To determine through econometric analysis which variables —inflation, real exchange rate, unemployment, and consumption— have a major impact on workers' wages and, therefore, on production.

Design/Methodology/Approach: We developed a multiple linear regression model for the behavior of macroeconomic variables in Mexico from 1995 to 2018, using the ordinary least squares method (OLS) and the Gretl statistical package.

Results: The analysis of the model showed that inflation, exchange rate, and unemployment are highly significant, unlike consumption. For the model of real wages in Mexico, we obtained a 0.87 coefficient of determination —*i.e.*, the variables included in the model account for 87% of the wages' behavior. The relation of consumption, unemployment, and inflation to wages was as expected. Regarding the exchange rate, the result was the opposite of the expectations. The wage-unemployment elasticity had the greatest impact.

Study Limitations/Implications: The database used was the main limitation because it relies on official sources, which lack data and show inconsistencies.

Findings/Conclusions: The study helped to determine whether or not the proposed variables affected the national economic growth. Mexico is not a first-world country than can offer high salaries; therefore, the Mexican economy must continue to grow, before it reaches a higher *per capita* income. In this regard, it is essential to consider the extent to which the new government's proposals will be able to face the reality: very few and very low-quality jobs are created, despite what the official figures say.

Keywords: Minimum wage, inflation rate, unemployment rate, exchange rate.

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INTRODUCTION

From the point of view of economics, wages are essentially a price: the price of subordinate labor. They make a distinction between nominal wages —which can be defined

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as "the flow of income in a currency that an individual receives"— and real wages —which refer to "the flow of goods and services that an individual may acquire with a monetary wage once the effect of inflation is deducted" (Buen and Valenzuela, 1997). According to the International Labour Organization [ILO] (OIT, 2020): "From the standpoint of economy, wages are an important part of labor costs and an essential variable for business competitiveness that needs to be analyzed, along with its relation to other factors like employment, productivity, and investment". Until 2014 the need to raise minimum wages was not understood. Nevertheless, implementing such a measure immediately or through a presidential decree could cause an endless inflationary spiral.

Consistent with the new wage policy, in 2019, the Mexican government reached for the second consecutive year the necessary consensus with the labor and business sectors, benefitting millions of workers (Secretaría del Trabajo y Previsión Social, 2019). In 2020, a 20% increase in the minimum wage resulted in $123.22 pesos per day. However, despite increases in recent years, this wage is still one of the lowest in the world. According to a study implemented by the global discount portal for online purchases Picodi.com, Mexico ranked 40th out of 54 countries, attaining a level similar to that of Vietnam (INFOBAE, 2020).

According to Aguirre (2020): "low wages favor exports at the expense of the purchasing power of workers. With better salaries, the virtuous circle of domestic consumption would have vastly improved this country's economy and increased tax collection".

The impact of an exogenous increase in minimum wages could have negative consequences on the overall economy, beyond inflation which would involve changes in the opposite direction. In particular, an increase in minimum wages that does not take into account the overall economic conditions would have redistributive effects. While some workers who earn the minimum wage would have a higher income, others could lose their formal employment. Moreover, this effect would intensify on those workers who could be potentially hired by companies. Additionally, the disintegration of the labor market into the informal sector could result in lower productivity. This diversion toward informality would negatively affect the development of physical and human capital and the adoption of new technologies, which, in turn, would result in lower growth in the future. A lower economic growth and higher informality would reduce the potential tax collection of the country, which would stress public finances (Banco de México, 2016a).

To minimize the negative economic consequences, the economic agents needed to understand that an increase in minimum wages does not imply that their expectations will be harmed. Therefore, increases were necessarily moderate and interpreted not as an adjustment in the cost of living, but as a change in the relative price of the workforce with lower earnings (Banco de México, 2016a).

In 2017, minimum wages increased by 9.58% (from $73.4 to $80.04). Considering the inflation for that year (6.77%), the purchasing power achieved a small gain (2.5%). In 2018, minimum wages increased by 10.39% and Banxico managed to reduce and control inflation at 4.83%; consequently, the purchasing power increased by more than 5%. In 2019, minimum wages increased by 16.21%, and inflation reached 2.83% —a >13% increase in purchasing power. The real annual salary growth results from the difference between wages and inflation. For example, if inflation reaches 5% and the wage increase is 8%, then the difference would be 3%, which is the real increase in purchasing power (Banco de México, 2016b).

The decrease in purchasing power can be clearly determined from the current inflation/minimum wage relation. This decrease may seem to render purchasing power less representative or important. However, when considered in its strictly real aspect, it becomes very relevant. Unlike other economic indicators, the unemployment rate is expressed in terms of people —*i.e.*, the number of persons that do not have a job at the time of the survey but who are looking for one (Gómez, 2014).

MATERIALS AND METHODS

For this research, secondary sources were used and a database with the annual data for macroeconomic variables in Mexico from 1995 to 2018 was developed, using constant prices from 2010. The information came from World Bank and INEGI databases.

An econometric approach was employed because it enables the development of models that help to analyze relations in economic theory, explaining the behavior of one variable in terms of others. Log-Log models where the elasticity of Y concerning X is attributed to $\beta 1$ were used and interpreted as a 1% increase in X related to a $\beta 1$% change in Y.

An econometric model is an economic model that includes the necessary specifications for its empirical utilization. The development of the econometric model included three stages. First, we specified the hypotheses to measure the phenomenon; then we estimated the values through data analysis, and we contrasted the established hypotheses; and finally, we evaluated the estimates based on economic, statistical, and econometrical criteria. A statistical contrast —between correlation coefficient and contrast tests— was carried out to check if the analyzed data and the results were significant, in order to determine not only the existence of the relation, but its nature.

According to Díaz and Llorente (2013): "Based on the sources of information, we established the relations between the variables considered the most relevant for the explanation of the behavior of the dependent variable. In most cases, economic theory does not explicitly establish the mathematical form of economic relations. Determining the function that links the variables considered in the analysis will clarify which type of relation exists between them. Subsequently, such knowledge will constitute the basis that justifies the adoption of economic measures". The ordinary least squares method (OLS) was used to obtain the estimators. When the function is well specified, one can make a correct estimate that takes into consideration different factors, such as the properties of the estimators, the object of the economic research, etc. (Gujarati, 2010). Estimation is a strictly technical stage that requires knowledge of various econometric methods, their operational hypotheses, and their economic implications (Díaz and Costa, 1994).

Wage functional relation (S_t)

There is an understandable concern in Mexico to improve the well-being of the low-income population, especially those living in poverty, hence the implementation of public policies that seek to increase the income of the most vulnerable. Other methods that help to reduce poverty include employment and training programs, outreach programs, and quality of public health and education services.

Wages are one of the most important variables for the country, since they reflect the population's overall purchasing power, which highly impacts production.

The wage functional relation in Mexico is calculated using the following formula:

$$LNS_t = f\left(LNTC_t,\ LNINF_t,\ LNC_t,\ LNU_t\right)$$

Where: LNS_t=Natural logarithm of wages in Mexico (pesos per day); $LNTC_t$=Natural logarithm of exchange rates in Mexico (peso-dollar); $LNINF_t$=Natural logarithm of annual inflation in Mexico (percentage); LNC_t=Natural logarithm of actual total consumption in Mexico (millions of dollars); LNU_t=Natural logarithm of unemployment rate in Mexico.

Structurally:

$$LNS_t = \pounds_0 + \pounds_1 LNTC_t + \pounds_2 LNINF_t + \pounds_3 LNC_t + \pounds_4 LNU_t + \xi 1$$

Economic production directly affects wages. When production increases, wages also increase and therefore purchasing power increases. Consequently, there must also be a direct relation with consumption since more goods can be acquired when wages go up.

As for unemployment, the contrary result is expected, because the increase in minimum wage will cause a decrease in unemployment. Regarding inflation, a direct relation is expected since the price instability erodes the purchasing power of people and inhibits economic growth and development. Finally, a direct relation with exchange rates is expected, because, in practice, currency appreciation causes an increase in wages, leading to more purchasing power and more consumption.

RESULTS AND DISCUSSION

The Gretl statistical package (2020) was used to analyze the real wages model in Mexico. First, the statistical results based on the parameters of the resulting equations were analyzed. Then the economic results based on the coefficients and their relation to the estimators of economic theory were analyzed. Finally, the results were analyzed based on the resulting elasticicities.

Statistical results

Table 1 was developed with the output of the Gretl software. The following variables were very significant: inflation (calculated t value: −8,027), exchange rate (calculated t value: −5,232), and unemployment (calculated t value: −3,210). Meanwhile, consumption was not significant (calculated t value: <1).

Regarding the equation for real wages in Mexico, the coefficient of determination has a value of R^2=0.87, meaning that the variables included in the equation account for 87% of the econometric model. In other words, 87% of the variations in wages were influenced

Table 1. Analysis of variance of the real wages model.

	Coefficient	Standard deviation	t-statistic	p-value	
Constant	11.3665	1.20505	9.432	<0.0001	***
l_C	0.0222138	0.0555262	0.4001	0.6936	
l_INF	−0.387036	0.0482154	−8.027	<0.0001	***
l_TC	−1.37172	0.262177	−5.232	<0.0001	***
l_U	−0.508974	0.158534	−3.210	0.0046	***
*** Denotes significance at 1%; ** Denotes significance at 5%; *Denotes significance at 10%					
Mean dependent variable		3.873694	D.T. of the dependent variable		0.364615
Sum of squares of residuals		0.332519	D.T. of regression		0.132291
R-squared		0.891252	R-squared corrected		0.868358
F (4,19)		38.92915	p-value (the F)		6.64e-09
Log-likelihood		17.29483	Criterion of Akaike		−24.58965
Criterion of Schwarz		−18.69938	Criterion of Hannan-Quinn		−23.02696
rho		0.189972	Durbin-Watson		1.503411

Source: Developed by the authors based on the GRETL output.

by the changes in consumption, inflation, exchange rates, and unemployment recorded in the country during the analysis period.

Finally, as seen in Table 2, the model lacks heteroscedasticity. In short, it presents significant variables and complies with the normality of residuals. Therefore, it is an adequate model.

Economic results

The analysis of the structural coefficients makes clear the relation between estimators and economic theory, which is implicit in the following equation:

$$S=11.3665+0.0222138C-0.387036INF-1.37172TC-0.508974U$$

Consumption and wages had the expected relation. When this variable increased, wages increased too, and *vice versa*. The opposite was true regarding the exchange rate: there is an inverse relation, rather than the one expected. The inflation-wages relation shows that,

Table 2. Contrasts in the real wages model.

Contrast of normality of the residuals- Null hypothesis: [The error has a Normal distribution].	Contrast statistic: Chi-square (2)=0.422621 con valor p=0.809522
Contrast of the specification RESET- Null hypothesis: [The specification is adequate].	Contrast statistic: F(2, 17)=5.95588 with p-value=P(F(2, 17)>5.95588)=0.0109568.
Contrast statistic: F(2, 17)=5.95588 with p-value=P(F(2, 17)>5.95588)=0.0109568.	Contrast statistic: LMF=0.821939 with p-value=P(F(1, 18)>0.821939)=0.376591
Contrast of the heteroscedasticity the White- Null hypothesis: [No heteroscedasticity].	Contrast statistic: LM=17.1161 with p-value=P(Chi-square (14)>17.1161)=0.250043

Source: Developed by the authors based on the GRETL output.

when inflation decreases, wages increase and vice versa, corroborating the hypothesis. The unemployment variable also behaved as expected.

An analysis that rigorously followed theory could show that increasing wages would be highly counterproductive. The minimum wage is not generalized: just a percentage of the population earns it. The current wage (W) is very different from the real wage, since workers are interested in what they can buy with their salary, not in the amount they earn —in other words, the wage:price (W:P) ratio, which is also known as purchasing power. An increase in W (*i.e.*, the current wage) causes an increase in consumer prices, basic consumer goods, and fuels. An increase in W can even affect all goods, which can cost more than they did before the current salary was raised, causing short and medium-term inflation, unless several fiscal or monetary policies are implemented. If we really want to increase wages over prices, the following conditions are required: higher productivity, appreciation of the peso, a stable exchange rate, and economic growth and development.

Economic interpretation of structural elasticities

Elasticity is a fundamental concept in economics: the percentage change in one variable (Y) in response to the percentage change in another (X). Most specifications consider that elasticity is not constant, since it depends on the concrete values of the explanatory variable (X) and the response variable (Y). Transformations applied to the variables affect the expression of elasticity (Alonso, 2009). The concept of *ceteris paribus* is essential for the analysis of elasticities in any given model; this concept enables the study of a variable isolated from the rest, in order to best observe how it changes when the other variables remain constant. Regarding wage elasticity, with a 10% increase in consumption, wages would rise by 0.20%. In the case of inflation, wages would decrease by 3.8%. Regarding exchange rates, wages would decrease by 13%. Finally, a 10% increase in the unemployment rate would mean a 5% decrease in wages.

Wages and employment are essential for development and social stability in any country, since labor with fair, decent, and sufficient wages guarantees a good quality of life among the population. However, labor is fractured in Mexico, because working conditions are precarious. The economy gravitates toward informality which creates few jobs or jobs that do not offer a decent salary or economic or social benefits and do not guarantee access to social security or quality education. A potential, yet very interesting contradiction arises: on the one hand, few formal jobs are created, but, on the other hand, according to unemployment statistics, the situation is better in Mexico than in European countries or in the United States. The low unemployment rates are a result of the way unemployment and informal employment are measured.

CONCLUSIONS

Fair wages are a sign of a healthy economy, but that is not the case in Mexico. For the same reason, the minimum wage has been raised gradually over the years. This increase depends on productivity, balance of payment, GDP *per capita*, and job supply and demand.

The databases used imposed some limitations on this research. Developing a longer-term database could make it easier to find and analyze an econometric model.

Nevertheless, the regression had an acceptable coefficient of determination. Although it was useful for the analysis and explanation of the behavior of the study variables, new variables can be introduced in future research, in order to enhance the explanatory power of the model. The following variables can be included to obtain better results: United States wage, number of migrants, and education level.

Unemployment, consumption, and inflation yielded results consistent with the hypotheses (*i.e.*, direct relations in each case). In contrast, the exchange rate did not behave according to our proposal and presented an inverse relation.

To conclude, we must understand that we are not yet a first-world country that can offer high salaries. Several goals must be met before that happens. We must wait for the Mexican economy to continue growing before a high *per capita* income can be achieved. In this regard, it is essential to consider the extent to which the new government's proposals will be able to face reality: very few and very low-quality jobs are created —despite what the official figures say.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Horseradish tree (*Moringa oleifera*) as a food product: value chain in the island of Ometepe, Nicaragua

Durán-García, Héctor M.[1]**; Ortiz-Laurel, Hipólito**[2*]**; Rössel-Kipping, Erich D.**[3]**; Pulido-Delgado, José L.**[1]

[1] Universidad Autónoma de San Luis Potosí, Instituto de Investigación de Zonas Desérticas, Altair No. 200, Col. del Llano, San Luis Potosí, San Luis Potosí, México, C.P. 78377.

[2] Colegio de Postgraduados, Campus Córdoba, Carretera Córdoba-Veracruz km 348, Congregación Manuel León, Amatlán de los Reyes, Veracruz, México, C.P. 94953.

[3] Colegio de Postgraduados, Campus San Luis Potosí, Iturbide No. 73, Salinas de Hidalgo, San Luis Potosí, México, C.P. 78600.

* Correspondence: ortizlaurelh@gmail.com; hlaurel@colpos.mx

ABSTRACT

Objective: To establish a local market and generate the value chain of horseradish tree (*Moringa oleifera*) as a food product to produce dietary supplements.

Design/Methodology/Approach: We propose a strategy to introduce horseradish tree to the island of Ometepe, Nicaragua, based on the concept of value chain analysis and the characteristic of sustainability. Four analysis aspects were taken into consideration: a) production, b) sustainability, c) socioeconomic, and d) political. Likewise, requirements and barriers to the introduction of horseradish tree as a sustainable food product were identified.

Results: Using synthetic fertilizers or pesticides is not recommended for the commercial production of horseradish tree leaf powder. From the polyculture farming of horseradish tree alone a $7,500-$8,000 USD yield in a 1-ha plot has been projected in the local economy for the first year and between $14,200 and $15,200 USD in the following years.

Study Limitations/Implications: Although the horseradish tree contributes to an improved nutrition, the consumption of the fresh parts of this species involves a change in the food culture.

Findings/Conclusions: Horseradish tree leaf powder is a viable alternative organic food and has the potential to respond to malnutrition in Ometepe, Nicaragua. It also represents a net profit between $5,050 and $5,600 USD in the first year and between $11,200 and $12,200 USD in subsequent years.

Keywords: Horseradish tree, horseradish tree powder, food value chain, food diet.

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INTRODUCTION

Horseradish tree is a species also known as moringa, drumstick tree, ben oil tree, and benzolive tree. It is a fast-growing, medium-sized, drought-resistant tree that is widely cultivated and naturalized in tropical and subtropical regions of the world. It is native to

Image by Iskandar Ab. Rashid at Pixabay

the sub-Himalayan regions of India, Pakistan, Bangladesh, and Afghanistan, where it has been traditionally used as a medicinal and nutritional plant (Radovich, 2011). This tree belongs to the monogenetic family Moringaceae, of which *Moringa oleifera* Lam. is the most known and used species (Nadkarni, 1976).

This tree has a short lifespan (around 20 years). Its height can range from 5 to 10 m and it is considered a small- to medium-sized tree (Reyes-Sánchez *et al.*, 2006; Reyes-Sánchez and Mendieta-Araica, 2017). It has trippinate compound leaves with 1-2 cm long, large, green to dark green, elliptical leaflets. It has 10-25 cm long, white to cream flowers. The horseradish tree fruit is a three-lobed capsule called a pod. The immature green pods turn brown as they mature and dry at the end of their cycle. Each pod contains 15-20 large seeds with paper-like wings.

This plant has recently gained importance due to its multiple uses and benefits. All parts of the horseradish tree are valuable and can be used to produce fertilizers, animal feed, dietary supplements, medicines, cosmetics, and biodiesel, as well as to purify water (Radovich, 2011). Studies have been carried out to identify its nutritional and medical values (Moringa Delight, 2020). The highly nutritious leaves, fruits, flowers, and immature pods are eaten as vegetables. The leaves are rich in β-carotene (vitamin A), protein, vitamin C, calcium, iron, and potassium, and they also are a source of natural antioxidants (Siddhuraju and Becker, 2003). Several case studies have been carried out to determine the nutritional value of fresh and dried horseradish tree leaves in multiple countries (Canett-Romero *et al.*, 2016); however, these values showed variations attributable to such factors as the analysis method, genetic background, and environmental conditions, as well as the cultivation method and its processing (CSA-FAO, 2018).

Regardless of the results, horseradish tree is believed to offer a local and profitable response to malnutrition in the tropical and subtropical regions of the world. It is particularly useful in those regions, because the leaves appear at the end of the dry season, when some other sources of green leafy vegetables are still available (Bonal-Ruiz *et al.*, 2012). Once dried, horseradish tree leaves can be kept for a long time, the cost of storage and processing is low, and they can be available all year round (Kar *et al.*, 2013). Horseradish tree production costs are low, it is grown in hot climates, it tolerates poor quality soils with a lack of water, and it is resistant to most pests and diseases (Kar *et al.*, 2013). As a consequence of the abovementioned characteristics, horseradish tree is of great interest to some non-governmental organizations (NGOs), which promote its multiple benefits. These NGOs promote its cultivation, local marketing, and sustainable consumption on the island of Ometepe, Nicaragua, where it is grown but not consumed.

There is scant information about the various processes, from cultivation, management, and harvest, to marketing, consumption, and health and economic benefits for local communities. Therefore, the objective of this work was to design a strategy aimed at establishing a local market and generating value chains that allow the community to obtain multiple benefits from the tree.

MATERIALS AND METHODS

The study area was limited to the island of Ometepe, located in Lake Nicaragua, in southern Nicaragua (11° 23' 00" and 11° 36' 00" N and 85° 26' 00" and 85° 43' 00" W) (Figure 1). This lake is part of a long tectonic depression that extends in the center of Central America, comprising an extension of about 500 km, from the Gulf of Fonseca in the Pacific Ocean to the alluvial valley of the San Juan River in the Caribbean Sea. The lake has an area of 8,264 km^2, which includes approximately 310 islands and islets. Ometepe is the largest of such pieces of land (UNESCO-MENR, 2010).

The study for the introduction of horseradish tree was based on the concept of value chain analysis, which has been accepted as an important tool for environmental research and development. Importance was attached to four aspects: a) production, b) sustainability, c) socioeconomic, and d) political. Likewise, existing requirements or barriers to the introduction of horseradish tree as a sustainable food product were identified. Similarly, information about the volume of production, losses, costs, sale price, required quality, sale regulations, taxes, and policies that impact the product was collected.

RESULTS AND DISCUSSION

The planning of this project required a high investment cost, especially to obtain access to a land plot, which was only possible through the involvement of an NGO. Likewise, to increase horseradish tree production, investment costs must be reduced; otherwise, additional financing must be obtained. Similarly, investment costs of the raw material processing must be reduced, production capacity must be increased, and production must be diversified.

To identify the production profitability, the market price of the final products must be incorporated. However, this information is not available at the study site. Therefore, the market price of powdered horseradish tree leaf in other countries is taken as a reference and tourists from those countries are asked how much they would be willing to pay for

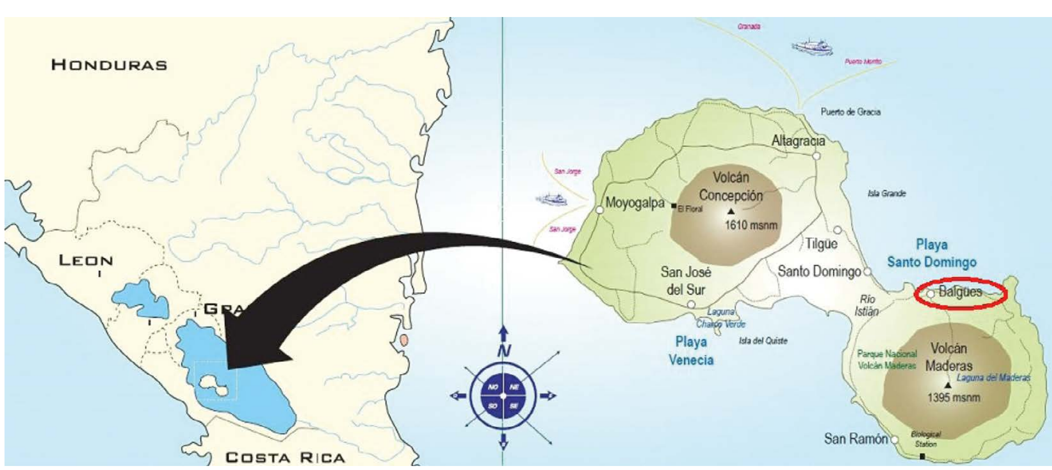

Figure 1. Location and map of the island of Ometepe, Nicaragua (Nicaragua-Conociendo Nuestro País, 2020).

200 g of the said product (Figure 2). Based on these results, the price of 200 g of powdered horseradish tree leaf was set at $5 USD.

Residents considered that the diversity of responses meant that it was an inadequate consultation procedure. It is assumed that, for this sector, the sale price must be lower since it will be produced locally and will be available directly from the producer and the local sales outlets.

Production

In general, three different ways of cultivating horseradish tree were found, which differ in the destination of some of the products to be marketed or the main purpose of the tree. The first was aimed at the intensive production of leaves. The second includes the complete development of the tree and is often known as a pod production method that also allows the production of leaves, but in smaller quantities. The third consists of a polyculture cultivation.

Based on the cultivation of 1 ha of land, a marketing pilot project was established; once the product is successful, the project can be expanded. However, given the lack of an agronomic program for its cultivation, the following assumptions were established based on the mapping carried out: a) establishment of polyculture with a 2×3 m configuration, resulting in a density of 1,650 trees per ha; b) use of locally collected seeds; c) use of manure and mulch, as the main sources of fertilization; d) manual pest management; e) three cuts in the rainy season and one in the dry season, during the first year; f) five cuts in the rainy season and one in the dry season, during the second year; and g) transportation of the product on horseback.

A passive solar dryer was used for processing and grinding was carried out with mechanical equipment. Fifteen sale points were considered, including stores and drugstores, with products mainly aimed at local people in 10 communities and 15 sale points exclusively aimed at tourists. An external motorcycle service was used to transport the final product to the sale point.

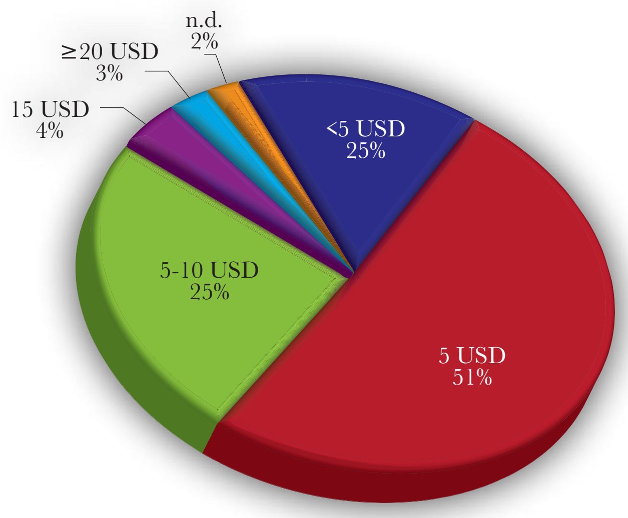

Figure 2. Price that tourists would pay for 200 g of powdered horseradish tree leaf. n.d.=not defined.

Sustainability

The introduction of horseradish tree as a food product has both positive and negative environmental aspects.

The positive environmental aspects are: a) horseradish tree cultivation offers the possibility of improving existing agricultural practices through the use of agroecological practices (*e.g.*, promoting the production of compost and reducing the use of petrochemicals); b) horseradish tree cultivation requires few inputs, no synthetic fertilizer, pesticides, or irrigation system are necessary, and, overall, manure as organic fertilizer is only required the first year during planting; c) horseradish tree leaf powder can potentially become a certified organic product; d) the promotion of polyculture in the study area provides environmental benefits such as weed suppression, reduction of insect damage through an improved balance of pests, better use of soil nutrients, and erosion control; and e) the production of horseradish tree powder requires a low input of electricity, since everything is done with passive solar energy.

The negative environmental aspect is the difficulty to reuse or recycle the waste from the production of horseradish tree, which poses an environmental and health risk.

Socioeconomic aspects

Horseradish tree polyculture diversifies the income, allowing farmers to become independent from the single-crop market. Higher income generation per hectare is possible, considering that horseradish tree can be harvested during the dry season. In addition, horseradish tree production reduces the dependence on imported dietary supplements, which implies employment creation and added value to the local economy. Horseradish tree has the potential to alleviate malnutrition in the study area and eventually promote a healthier behavior in local eating patterns. Over time, other uses can emerge, such as forage for animals.

Political aspects

There are no current health and quality standards for the production of horseradish tree leaf powder, no system monitors compliance with agricultural regulations, and the waste management system in the area is inadequate. Therefore, greater political intervention is required to reduce the negative environmental and social impacts of horseradish tree leaf powder production and to promote general economic development.

CONCLUSIONS

Given the current conditions on the island of Ometepe, Nicaragua, the powdered tree leaf represents a more viable alternative for the consumption of horseradish by local communities and foreign tourists through nutritional supplements. To make its consumption more attractive, an organic horseradish tree production program can be implemented. However, there are no quality certification and health standards for the consumption of horseradish tree leaf powder. The investment required to establish a horseradish tree plantation is high and there is no state funding or tax benefits in the horseradish tree value chain, despite its outstanding position as a sustainable niche market and employment generator. In addition, a hectare of horseradish tree could add to the local economy between $7,500 to $8,000 USD in the first year and between $14,200 and $15,200 USD in the following years. The net profit in the first year would amount to $5,050-$5,600 USD and $11,200-$12,200 USD in subsequent years.

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AGRO PRODUCTIVIDAD
Colegio de Postgraduados

Germination and growth of the Sonoran Desert native trees under different agricultural conditions

Ochoa-Meza, Andrés[1]; Montiel-González, Cristina[2]; Ruelas-Islas, Jesús del Rosario[3]; Barrera-Silva, Miguel A.[1]; Valdez-Domínguez, Ramón D.[1]; Celaya-Michel, Hernán[1*]

[1] Departamento de Agricultura y Ganadería, Universidad de Sonora, Carretera a Bahía de Kino km 21, Hermosillo, Sonora, México, C. P. 83000.
[2] El Colegio de la Frontera Sur, Av. Rancho Polígono 2A, Parque Industrial Lerma, Campeche, Campeche, México, C. P. 24500.
[3] Facultad de Agricultura del Valle del Fuerte, Universidad Autónoma de Sinaloa, Calle 16 s/n esq. Japaraqui, Juan José Ríos, Ahome, Sinaloa, México, C. P. 81110.
* Correspondence: hernan.celaya@unison.mx

ABSTRACT
Objective: To evaluate the germination, survival, and growth of Sonoran Desert native trees, in a fertile agricultural soil, with irrigation and protection against herbivory.
Design/Methodology/Approach: For six months, the germination, survival, and growth of five tree species (*Parkinsonia microphylla*, *Olneya tesota*, *Prosopis velutina*, *Guaiacum coulteri*, and *Parkinsonia florida*) were evaluated in agricultural soil, with and without drip irrigation, avoiding herbivory.
Results: The irrigated species *O. tesota* and *P. velutina* had the highest germination percentage (χ^2=398.941, p<0.0001). Plant survival was above 62% (χ^2=21.196, p<0.0035), except for *G. coulteri* and *P. florida* without irrigation, which did not survive. At six months, *P. florida* recorded the greatest height (p<0.0001), while all the species without irrigation and *G. coulteri* with irrigation recorded the lowest heights. Likewise, *P. velutina*, *P. florida*, and *O. tesota* (p<0.0001) registered the greatest canopy cover at six months, while non-irrigated plants of all species and irrigated *G. coulteri* had the least cover.
Limitations/Implications: Further agronomic studies are necessary to enable the successful establishment of commercial forest plantations, increasing knowledge about environmental problems.
Findings/Conclusions: The five species of native plants studied can be established by direct sowing, in agricultural soil and with drip irrigation.

Keywords: Forest plantations, ecological restoration, ecosystem services.

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INTRODUCTION

The state of Sonora, in northwestern Mexico, has a highly diverse flora composition (Van Devender *et al.*, 2010). Most of the state is located within the Sonoran Desert (Shreve and Wiggins, 1964) and there is still a great lack of knowledge about its biodiversity. For example, the growth potential of various plant species has not been yet documented (CONAFOR, 2010). In arid and semi-arid zones, plant growth is slower than in humid zone ecosystems. Growth limitation is the consequence of two key factors that determine


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both the structure and the function of these ecosystems: firstly, the low availability of water for plants (Noy-Meir, 1973; Zhou, 2009) and, secondly, the low availability of nutrients in the soil (Whitford, 2002). As a third stressor, herbivory also interferes with plant growth and survival in these natural ecosystems (Bainbridge *et al.*, 1993; Ward, 2009).

The biodiversity of the Sonoran Desert is not exempt from current global challenges, such as overexploitation, climate change, and desertification (Van Devender *et al.*, 2010). Despite this worldwide problem, the restoration of degraded lands has not been extensively studied in arid and semi-arid environments (Bainbridge *et al.*, 1993). An alternative to partially mitigate the current environmental problems is the establishment of commercial forest plantations, which help to recover degraded land, capture carbon, and obtain satisfiers from ecosystems in a sustainable manner (CONAFOR, 2010; Li *et al.*, 2012).

Most of the experiments with native desert plants are carried out with local rainfall and some with a small supplementation of irrigation water (Bashan *et al.*, 2012), but not with drip irrigation. This technology increases the efficient use of water in ecosystems where this resource is limited, avoiding loss through evaporation and guaranteeing the availability of water for plants (Taylor and Zilberman, 2017). Few studies about the revegetation of deserts with native plants use irrigation as a treatment; even less studies use drip irrigation and few mention the height and canopy growth of the plants studied (Hessing and Johnson, 1982; Cox *et al.*, 1984; Abella and Newton, 2009; Woods *et al.*, 2012; Taylor and Zilberman, 2017).

Most of the studies about the forest species development in arid zones have been carried out in Africa or Asia (Duku *et al.*, 2016; Farahat and Linderholm *et al.*, 2015; Jiao *et al.*, 2021), where the possibilities of irrigation with urban effluents were analyzed, as an opportunity to reuse water. However, it is very important to determine the growth potential of native plants to help decision-makers in charge of ecological restoration programs or to establish commercial forest plantations; in both cases, obtaining more ecosystem services from arid and semi-arid zones would improve the balance between environmental benefits and those of landowners (Wormald, 1995; CONAFOR, 2010; Djanibekov *et al.*, 2012; Boreux *et al.*, 2013; Sánchez-González *et al.*, 2017; Zeng *et al.*, 2018).

The objective of this work was to evaluate, during a six-month period, the germination, survival, and potential growth of the Sonoran Desert native trees in an agricultural soil and with drip irrigation; additionally, the said trees did not face the water availability limitation of natural ecosystems and they were protected from herbivory.

MATERIALS AND METHODS

The experiment was developed in the Experimental Field of the Departamento de Agricultura y Ganadería of the Universidad de Sonora (DAG Unison), in Hermosillo, Sonora, Mexico, which is located at 29° 00' 55" N and 110° 07' 59" W. The mean annual temperature is 25.2 °C. The maximum temperature is registered in the month of June (39-41 °C) and the minimum is registered in the month of January (7-10 °C). The average annual rainfall is 378 mm, with August being the wettest month (100 mm) and May, the driest month (2.5 mm) (INEGI, 2014; SMN, 2019). The experimental agricultural area consists of an open field, from which fauna and domestic animals are excluded.

The main physical and chemical properties of the soil were determined up to a 30-cm depth (Table 1). Although the fertility in arid ecosystems is exceptionally low, no fertilizer was added (Cox *et al.*, 1984; Whitford, 2002).

Germination in agricultural soil

Seeds of five native plant species were collected at DAG Unison, with the proper collection permits from SEMARNAT (Table 2). One-hundred seeds of each species were germinated and maintained with drip irrigation. Likewise, in the same agricultural soil, 100 additional seeds of each species were sown and kept without irrigation; the purpose was to determine if they can germinate only with rainwater. The seeds were germinated directly in 40-cm wide raised furrows, separated by 1 m. One seed of each plant species was sown every 20 cm on 20-m long furrows. Sowing depth was twice the size of the seed. Sowing was carried out on June 4, 2018. The seeds were dry sown and then drip irrigation was applied to the raised furrows under the said treatment. A 1-cm irrigation sheet was applied per week. With $>$20 mm rainfall, weekly irrigation was suspended.

The study period comprised six months from June 2018. Seed germination was checked three days a week, dead seedlings were counted, and the possible cause of death was noted. Precipitation was recorded with a physical rain gauge in each rain event of the study period.

Experimental design

A bifactorial experimental design was planned, naming the species as the first factor and the irrigation treatment as the second factor. The levels of the first factor were the five native plant species. The levels of the second factor were drip irrigation and exclusively rainwater. One hundred repetitions per treatment were established.

At six months, the germination percentage, survival percentage, and height and width of the canopy were evaluated.

Table 1. Physical and chemical characterization of the soil.

pH	E.C. dS m^{-1}	O.M. %	NO_3^-	PO_4^-	K	Ca	Mg	S	Fe	Cu	Zn	Mn
			mg kg^{-1}									
7.73	0.56	0.20	17.6	132.4	126.0	1830.0	160.0	71.0	2.0	2.5	0.6	0.8

E.C.: Electric conductivity. O.M.: Organic matter.

Table 2. List of native plant species used in this study.

Common name	Scientific name	Seeds with irrigation	Seeds without irrigation
Guayacán	*Guaiacum coulteri* A.Gray	100	100
Palo fierro	*Olneya tesota* A. Gray	100	100
Palo verde azul	*Parkinsonia florida* (Benth. ex A. Gray) S. Wats.	100	100
Palo verde	*Parkinsonia microphylla* Torr.	100	100
Mezquite	*Prosopis velutina* Woot.	100	100

Plant canopy height and cover

Six months after germination, 25 plants were randomly sampled per species, except for some species where the total number of individuals alive was sampled, which was noted in the corresponding place. A tape measure was used to obtain plant height and leaf canopy width data.

Statistical analysis

Statistical analyzes were performed with the JMP version 10 software (SAS Institute, 2000). With the germination and survival data, contingency tables were developed using the chi-square test (χ^2) (Infante and Zarate de Lara, 2000). The rest of the information about the plants height and canopy cover was contrasted by analysis of variance. Tukey's test was used a posteriori. In all cases, a $\leq$5% statistical significance was established.

RESULTS AND DISCUSSION

Table 3 shows the germination and survival results. The five species showed germination, except *O. tesota* without irrigation. The species that had the highest germination percentage were *O. tesota* and *P. velutina*, both under irrigation with 85 and 72%, respectively. Meanwhile, *P. microphylla* with and without irrigation and *G. coulteri* with irrigation showed a 29-43% germination. The lowest germination values were found in the seeds under the non-irrigated treatment.

Table 3 shows that species with $\leq$15% germination values may require some type of seed scarification. This is the case of *P. florida*, whose seeds can last for several years in the soil before germinating, in the absence of scarification (Phillips *et al.*, 2015). Other authors report that different methods —such as mechanical scarification with sulfuric acid, temperatures alternation (Connor *et al.*, 2008), or immersion in hot water (Bainbridge, 2012)— benefit the germination of the seeds of genus *Parkinsonia*. Naturally, many species are consumed by animals that scarify or condition the seeds for better germination, after they pass through their digestive tract (Shreve and Wiggins, 1964; Whitford, 2002). Meanwhile,

Table 3. Germination (χ^2=398.941, p<0.0001) and survival (χ^2=21.196, p<0.0035) of the native plants studied.

Species	Treatment	Germination, %	Survival, %
G. coulteri	Without irrigation	4.00	0.00
O. tesota	Without irrigation	0.00	NA
P. florida	Without irrigation	1.00	0.00
P. microphylla	Without irrigation	29.00	65.52
P. velutina	Without irrigation	10.00	80.00
G. coulteri	Irrigation	43.00	62.79
O. tesota	Irrigation	85.00	65.88
P. florida	Irrigation	15.00	93.33
P. microphylla	Irrigation	43.00	83.72
P. velutina	Irrigation	72.00	79.17

new ironwood seeds germinate without problems (Shreve and Wiggins, 1964). *O. tesota* did not germinate without irrigation; this could be the consequence of the extraction of seeds by small rodents (Connor *et al.*, 2008) and insects such as ants, which has been observed in other studies (Celaya, personal obervation).

The rains in the study area recorded an accumulated value of 446 mm during the six months analyzed (Figure 1), which surpasses the average annual rainfall of 378 mm (INEGI, 2014). The highest precipitation was mainly concentrated during the months of July, August, September, and October, which are the months in which tropical cyclones take place. These extraordinary rains may have influenced the germination and survival of the species without irrigation and showed what happens in a year with above-average rainfall (Table 3). Therefore, the germination results in the non-irrigated areas of this work could be more severe in years in which droughts cause water stress.

Plant survival (Table 3) was higher than 62%, except for *G. coulteri* and *P. florida* without irrigation, which did not survive. In studies about *P. velutina*, *P. microphylla*, and *P. florida* in Baja California Sur, within the Sonoran Desert, irrigation was added up to the equivalent of 300 mm of rainfall per year. The annual survival rates surpassed 80% (Bashan *et al.*, 2012); these data are similar to those obtained in this study for the same three species. However, the survival expectancy without irrigation is only around 2% (Bainbridge *et al.*, 1993).

Herbivory damage was recorded during the six months of the study. When the first rains occurred, small rodents and ants extracted seeds —mainly of *O. tesota* without irrigation. Apparently, in the treatments with irrigation, seeds germinated faster, reducing the animals' opportunity to extract ungerminated seeds. After several months of growing, some plants were eaten by moles (Talpidae); this phenomenon was observed through the drying of the plant and total cuts at the stem base level. However, no more than 5% of the experimental population of *O. tesota*, *P. velutina*, *P. microphylla*, and *P. florida* died due to herbivory.

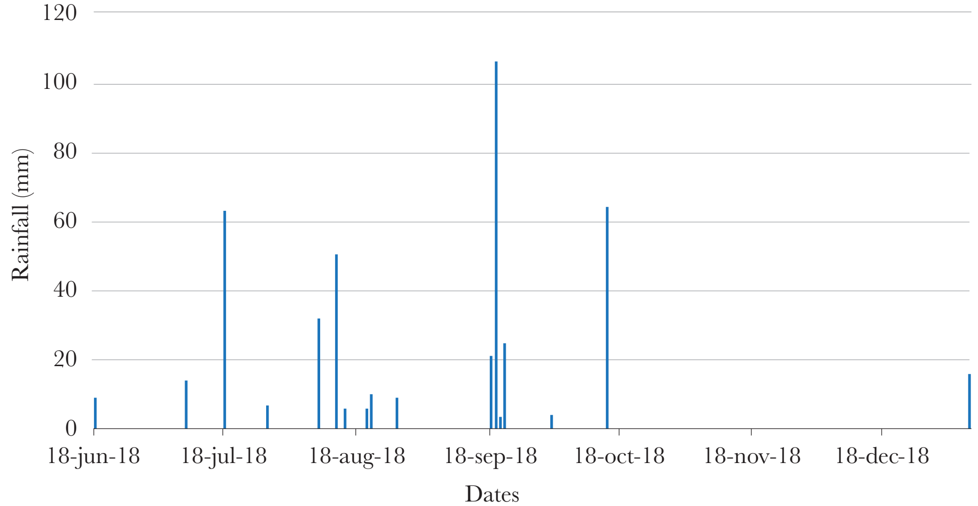

Figure 1. Precipitation during the study period in the Departamento de Agricultura y Ganadería, Universidad de Sonora (DAG Unison), in Hermosillo, Sonora, Mexico.

Growth rate

Table 4 shows significant differences in height at six months of age ($p<0.0001$) among the species studied. The greater height was presented by *P. florida*, followed by *P. velutina*, *O. tesota*, and *P. microphylla*, while the lowest heights were recorded in plants without irrigation and *G. coulteri* with irrigation.

A transplant experiment with drip irrigation, close to our study area, but established on non-agricultural soil, reports *Ipomoea arborescens*, *P. florida*, *P. microphylla*, *P. velutina*, and *O. tesota* as species with the highest growth in 1.5 years, with 1.41, 1.41, 1.33, 1.31, and 0.69 m height, respectively, and the lowest growth for *G. coulteri* with 0.38 m (Mc Caughey-Espinoza *et al.*, 2017). These results match the trends shown in Table 4.

In the Sonoran Desert, during the first year and with 300 mm of annual rainfall, *Prosopis* sp., *Parkinsonia microphylla* Torr., and *Parkinsonia florida* grew up to 35, 50, and 130 cm, respectively (Bashan *et al.*, 2012). The height data recorded in this study after six months are similar to the results obtained for the same species, with values of 66, 44, and 101 cm, respectively.

The highest canopy cover values at six months were presented by *P. velutina*, *P. florida*, and *O. tesota* (Table 5), while the lowest values were found in plants without irrigation and *G. coulteri* with irrigation. There was no statistical difference among the three species with the largest canopy diameter; they showed a lower height than *P. florida*. The species

Table 4. Height of the species studied after six months of growth ($p<0.0001$).

Common name	Treatment	Number	Height (cm)
P. florida	With irrigation	14	102.0±21.5 a
P. velutina	With irrigation	25	66.9±28.6 b
O. tesota	With irrigation	25	60.9±21.9 b
P. microphylla	With irrigation	25	44.6±12.7 c
P. microphylla	Without irrigation	13	23.3±7.8 d
P. velutina	Without irrigation	8	20.8±9.1 d
G. coulteri	With irrigation	25	6.2±1.8 d

Means ± SD with different letter in the height variable are statistically different, Tukey ($p<0.05$).

Table 5. Canopy cover (cm) at six months of growth ($p<0.0001$).

Species	Treatment	Number	Canopy cover (cm)
P. velutina	With irrigation	25	67.8±24.9 a
P. florida	With irrigation	14	67.1±8.8 a
O. tesota	With irrigation	25	56.7±14.7 ab
P. microphylla	With irrigation	25	43.9±19.0 b
P. microphylla	Without irrigation	13	23.5±9.2 c
P. velutina	Without irrigation	8	20.0±11.2 cd
G. coulteri	With irrigation	25	5.3±0.9 d

Means ± SD with different letter in height are statistically different.

without irrigation and *G. coulteri* obtained the lowest canopy width values; these same species had a similar height behavior at six months (Table 4).

Results in the Sonoran Desert have shown low success rates with direct seeding (Bainbridge *et al.*, 1993). However, this study shows that irrigation, agricultural soil, and protection from medium and large herbivores may have contributed to the high survival rates (Table 3).

Further and longer studies with more species of trees that take into account the impact of agronomic practices (*e.g.*, irrigation, fertilization, and pruning) would provide more elements for improved commercial forest plantations in arid and semi-arid zones (Bainbridge *et al.*, 1993; CONAFOR, 2010), both with timber and non-timber species within the plant biodiversity of the Sonoran Desert. This first six-month approximation provides a good prospect about the feasibility of establishing native species plantations. Likewise, we have observed the behavior of germination and growth in a year of atypical rainfalls. The importance of this event lies in the fact that some climate change scenarios predict that these rain events may become more extreme as the years go by, either towards droughts or heavy rains. Having information on the response of native species to environmental variations allows us to select those that can tolerate or benefit during these atypical years.

CONCLUSIONS

It is feasible to establish the five native plant species studied by direct sowing, in agricultural soil and with drip irrigation. Survival in the first months of growth is strongly influenced by the availability of water. Similarly, plant height and canopy cover were different between species, which is determined by their growth rate. Further studies are required to learn more about agronomic elements and about the growth potential of these and other native plants, which will enable better decisions about the convenience and resources necessary to establish commercial forest plantations.

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AGRO PRODUCTIVIDAD

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The self-management organization as a way for the *in situ* conservation of native poultry genetic resources

Romero López, Ana R.

Colegio de Postgraduados, Campus Montecillo. Carretera México-Texcoco km 36.5, Montecillo, Estado de México, México, C. P. 56230.
Correspondence: anarosa.romero.lopez@gmail.com

ABSTRACT

Objective: To identify local organization strategies employed by peasant women to conserve their native hens.

Methodology: Informal dialogues were held with five peasant women from the rural community of La Cuchilla, Nopala de Villagrán, Hidalgo, Mexico. The information collected was analyzed based on self-management organizational elements. Likewise, the proposals of three authors were used as a foundation for the analysis of the community organizational points in question, with the aim of strengthening the self-management organization.

Results: Peasant women use specific self-organization strategies to conserve their native hens, which are considered of great value as a result of their resistance to diseases and their adaptation to the local feeding and environmental conditions. Based on the findings and the authors consulted, an analysis framework is proposed to design of actions that strengthen the self-management participation of women in the conservation of their native poultry resource.

Study Limitations: Further studies about women self-management organization mechanisms in rural communities aimed at the conservation of their resources are recommended. The said studies will enable a more complex description of their functionality, potential, and cooperation mechanisms, among other elements that strengthen their structure.

Conclusion: The mechanisms of self-management organization have great potential for the local and community conservation of native poultry resources.

Keywords: poultry farming, self-organization, native hens, rural community.

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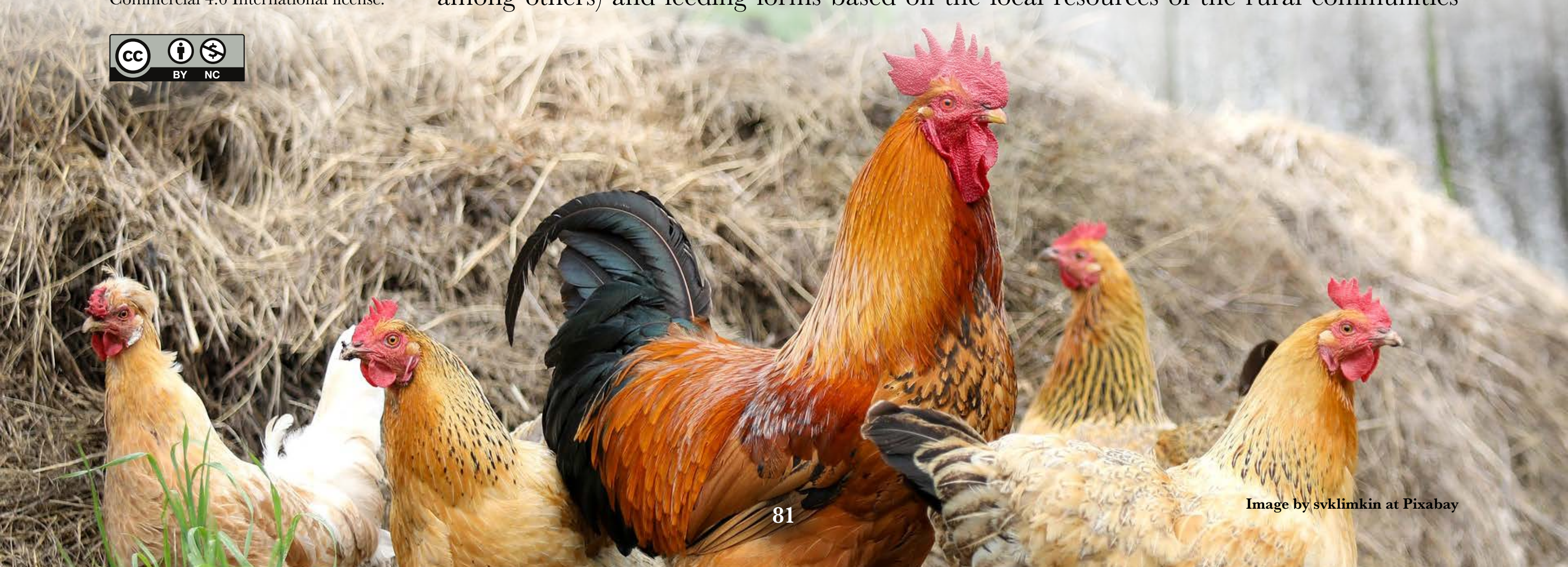
INTRODUCTION

As a result of natural selection and the selection made by the community, native poultry resources have adapted to environmental conditions (climate, soil type, presence of diseases, among others) and feeding forms based on the local resources of the rural communities



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where they develop. Consequently, native poultry have acquired characteristics that the people who use this kind of birds consider valuable (Camacho *et al.*, 2016). Likewise, these genetic resources have evolved together with the social, economic, cultural, and management knowledge of the people who have used them in a specific context. In other words, these resources have not only represented an accumulated genetic heritage, but also local lore that reflects the cultural and historical identity of the communities that developed them; these resources are an integral part of their life and traditions and they have co-evolved with an environment and concrete production system (FAO, 2010).

Nevertheless, several causes have contributed to the loss of these genetic resources. Among them, the uncontrolled crossbreeding between native animals and stocks —animals genetically selected to increase their production— stand out. These stocks have been introduced as part of projects or programs designed by external agents that have exacerbated comparisons between native breeds and stocks, diminishing the productive characteristics of breeds adapted to the local environment. In its turn, this situation has led to the transformation of traditional systems into systems oriented to the use of external inputs (Animal Production and Health Division, 1998; FAO, 2007).

Given this scenario, native breeds must be considered as valuable genetic material that needs to be maintained and improved, based on appropriate national policies and programs for genetic resources conservation. A low-cost alternative to achieve this conservation is in situ conservation —which consists of actions where the breed continues to occupy the environment in which it has been developed (Animal Production and Health Division, 1998).

In situ conservation measures highlight the need to recognize the value and importance of local production systems; these systems should be supported, given their importance for the maintaince of these zoogenetic resources (FAO, 2007). To achieve this objective, two measures are necessary: on the one hand, the elimination of factors that contribute to genetic erosion; and, on the other, the promotion of the exchange of ideas, the interaction and dialogue between indigenous and rural communities and scientists, public servant, and other stakeholders, in order to combine traditional lore with scientific approaches (FAO, 2010).

Not only the production systems *per se*, but also the people who develop them are considered important in the measures recommended for *in situ* conservation, since the lore, innovations, and organization of the farmer, indigenous, and local communities are valuable, pertinent, and necessary for the conservation and sustainable use of the domestic animal diversity (SAGARPA, 2002).

FAO (2010) has recognized the importance of the rural community members' lore and management for the *in situ* conservation of native genetic resources. However, the guidelines that it has proposed for conservation plans (Animal Production and Health Division, 1998) seem to have an approach from above (government) to below (community), which leaves aside the functionality and potential of the self-management organization methods of the people who use the resource and who make up the community (appropriators). For example, the proposals made by external agents fall into the following categories: 1) the creation of programs and strategies established by the government, 2) experts, 3) associations, 4) cooperatives, and 5) organizations that work with the community to conserve the native genetic material (Animal Production and Health Division, 1998). Consequently, these guidelines consider that the appropriators should be directed by a bureaucratic-like level and not by themselves.

Drawing an analogy with Ostrom's (2000) statement about the possibility of handling common use resources (CUR) through a self-management organization, we can glimpse viable ways for the conservation of native genetic resources, not only through external or bureaucratic agents, but also directly by the users of the resources themselves.

For example, Ostrom (2000) considers that a group of individuals could cooperate with each other and govern themselves to make a rational use of forest, fishery, or livestock CURs, without necessarily fulfilling the predictions of the tragedy of the commons, the prisoner's dilemma, and the logic of collective action —three models that indicate the impossibility of collective action and the unlimited use of resources in a limited world, where the objective is to maximize profits and satisfy self-interest.

This situation opens the possibility that self-management organization can also be considered as a way to conserve resources.

The objective of this exploratory study was to identify the existence of local organization strategies aimed at the conservation of native hens in a rural community.

MATERIALS AND METHODS

Study area

This exploratory research work was carried out in the La Cuchilla community, in the Nopala de Villagrán municipality, Hidalgo, Mexico. Informal dialogues were carried out with five women who had poultry in the community, in order to explore and learn about the value they assigned to native birds and their local forms of conservation and repopulation. The information collected was analyzed based on Ostrom's proposal (2000) about the elements that favor and strengthen the self-management organization. Based on Ostrom (2000), Wolf (1981), and Agarwal and Narain (1997), the organizational points to be considered for the community-level self-management organization were analyzed, including: 1) criteria for the consolidation of the interest group, 2) recognition of the appropriators' local lore, and 3) identification, recognition, and strengthening of binding contracts (local arrangements, system of rules).

RESULTS AND DISCUSSION

An example of a self-management organization for the conservation of native birds

The peasant women put great value on the Native hens as livestock element, because they have characteristics that fit the production system in which they have developed, including rusticity, resistance to diseases, and natural instincts such as brooding (an important characteristic for the natural reproduction of the species). Furthermore, native hens satisfy the needs of small producers, given the important use value that the community attributes to them. Native hens fulfill social (strengthening interpersonal ties between members of the family and the community), cultural (eggs and meat used in traditional medicine), economic (higher economic value than birds of improved genetic lines), nutritional (poultry products with characteristic and natural flavor), and recreational functions.

Despite their importance, these hens had been replaced by stocks to "boost" food production in rural communities, based on programs that have diluted the existing local genetic material. In response to this situation, some of the peasant women —who are part of the community and maintain bonds of friendship or family ties— organized themselves into a small group to protect their livestock resource and promote its reproduction through the following actions: a) do not include hens from government packages in their production unit, b) exchange among themselves native hens and roosters from the same community for reproduction purposes, and c) establish a mechanism for the incubation of native birds that consists of the collection of native eggs which were subsequently incubated by female turkey. Small producers who did not have female turkey in their production unit could take advantage of the following share cropper mechanism: a producer with female turkey would have them incubate the eggs of the producer that did not and the number of hatched chicks would be divided between the two producers.

The abovementioned data provide a glimpse of the interest of women in CUR conservation, through specific self-organized actions aimed at a common good.

In this sense, Ostrom (2000) mentions that the potential cooperation among a set of individuals for the rational use of CURs depends on a series of internal elements that influence the individual decisions about each subject: a) costs and benefits of the individuals' actions and their link with the expected results, b) CUR provision and appropriation over time, that is, the different activities required to maintain the resource, as well as their allocation (task and resulting resources division), c) the discount rate, that is, the value given to immediate or future benefits, and d) external elements, such as the reduction of uncertainty (lack of information). In the specific case of the handling of eggs from native hens —taking up again the elements proposed by Ostrom (2000), which stimulate cooperation between people—, it is possible to determine that the strategies established between women had their origin in the shared value given to the resource, as a way to solve problems that were perceived as such and in the interest of obtaining a long-term benefit from it.

Likewise, the established strategies indicated that resources were allocated according to the participation of each member: each woman obtained the resources that matched the activities that she had carried out. Ostrom (2000) also mentions that the creation of provision, credibility/trust, and supervision mechanisms can, in turn, favor and strengthen stable self-management institutions. In the case of this study, we were able to identify that the women who made up the group had a previous bond of trust, either because they were family members or friends.

In accordance with the proposals of Ostrom (2000), it is possible to elucidate that the users of the resource themselves can design the long-term and small-scale CUR-conservation actions, discarding the assumption that individuals are incapable of cooperating with each other for a common good (Ostrom, 2000).

In this sense, Palerm (2000) proposes the existence of two types of self-managed administration for the management of a CUR (such as water): 1) cases where the operation and other activities are carried out by the users of the resources themselves, and 2) cases where the users of the resource hire specialized personnel for the operation. The particular implications of each type of self-management are taken into consideration; for example, when the type of self-management is carried out by the users of the resource themselves, in a small-scale context, but limited by the needs of technical capacity and specialists, no type of bureaucratic administration is necessary. Making an analogy with the analysis and proposal of Palerm (2000), it is possible to determine that small-scale systems that involve peasant women who own poultry can be fixed by setting up a simple self-management organization and carrying out the tasks with the help of the members themselves. The previous theories demonstrate the importance of recognizing and making visible the self-management option of the CUR users themselves and valuing its innovation capacity (Palerm, 2000).

Organizational elements for the conservation of native poultry genetic material as a CUR

Based on Ostrom (2000), Wolf (1981), and Agarwal and Narain (1997), we propose the following organizational elements to design actions that strengthen self-management participation in the conservation of native poultry genetic material as a CUR at community level:

1. Criteria for the consolidation of the interest group

In order to determine the feasibility of the implementation of collective actions for the use and exploitation of a CUR (*e.g.*, native hens), the elements proposed by Ostrom (2000) for the consolidation of interest groups can be considered: a) the minimum number of participants necessary to obtain a collective benefit, b) participants with common or similar interests that encourage internal cooperation, and c) the interest that future generations obtain future benefits from the said resource. Consequently, the actions proposed for the reproduction, use, and exploitation in common of the native poultry resource can be considered and modified by the interest group to achieve its long-term conservation.

Unlike Wolf (1981) —who considered that the persistence of corporate organizations relied on tradition and obligation and that they sought to find a balance (evening out their opportunities and risks among their members, based on their internal function)—, we conjecture the possibility that stable self-management organizations have their origin in the existence of groups that can voluntarily organize themselves through self-organized collective actions. Therefore, the origin of these organizations may arise from their internal needs to regulate and maintain their resources, rather than from external pressures or any historical precedent.

2. Recognition of the resource users' local lore

The identification and recognition of the local lore about the CUR will provide information that will influence the actions and decisions that will be taken for its conservation. The foregoing will help to avoid affecting the discount rate, as well as reduce the uncertainty of the long-term existence of the resource. Therefore, the information obtained will allow the design of actions and technologies appropriate to a specific reality aimed at achieving the common benefit. In this sense, Agarwal and Narain (1997) consider that modern science and technology, along with the essence of high productivity, have led to the relegation of lore, traditional cultures, and ancient ecological rationality, resulting in technologies with high ecological costs. In this case, the dilution of genetic material (loss of biodiversity) is an example of a highly negative ecological impact.

Therefore, nowadays it is essential to develop technologies that are suitable for the appropriators, that respond to their needs, and that are consistent with the social, cultural, economic, and environmental context of the locality, in order to increase the feasibility of conserving a CUR (Agarwal and Narain, 1997).

3. Identification, recognition, and strengthening of binding contracts (local arrangements, rules system)

Taking Ostrom (2000) up again, the organization may last in the long term, not because they continue with a traditional model, but rather as a result of the function type and dynamics of its structure, whose rules do not remain static, but undergo dynamic changes, where a system of rules, trial, and error can be experienced. The organizational balance will be the result of their agreements and compliance with the rules, the arrangements reached between the individuals involved, and the possibility that those affected by the operating rules can participate in its modification.

Likewise, since the people interested in the conservation of a CUR created the rules themselves, they supervise each other as a result of their own interest, and consequently determine if the common good can be achieved through long-term joint work.

Face-to-face communication within the organization is necessary for the establishment and operation of binding contracts. It places a high and important value to the actions or strategies shared by others (shame, guilt, among others), which increases the probability of establishing coherent agreements that define the role and rewards of each individual. Consequently, the CUR is not overexploited in the process and there is a clear definition of the expected costs and benefits.

Reciprocity, trust, and communication are components that can favor and strengthen cooperation between individuals for the rational use of the CUR. Based on the approach proposed by Ostrom (2000) for the study and analysis of whether or not institutions successfully manage common use resources, a glimpse of the dynamics between individuals and the links they establish through reciprocity, trust, and communication can be achieved, as well as how, in turn, they build an organization that seeks to achieve a long-term common good.

Ultimately, the recognition, respect, and strengthening of the local arrangements established by the organized groups for the conservation of the CUR will allow greater control of the decisions and designs of their own contracts, along with equal opportunities to use the resource in question.

4. External recognition of the organization's rights

Although the existence of self-management organizations does not depend on any external authority, external authorities must recognize the minimum rights of the organizations. In other words, external government authorities should not question the rights of the appropriators to build their own institutions.

Actions proposed to strengthen self-management organizations that work for the conservation of native hens in the La Cuchilla community

To strength the self-management organization in La Cuchilla and taking into account the abovementioned organizational elements, it is proposed the following elements.

The previous proposal aims to make visible and strengthen the self-management organizations of women in the community, as a way to conserve their native hens. It should be emphasized that further studies about the ways in which peasant women in rural communities organize themselves for the conservation of their native poultry resources are required. These studies use an analytical framework —such as the one proposed in this study— to achieve a more complex description of their functionality, cooperation mechanisms, lore, binding contracts, and rules, among other elements, to determine

Table 1. Proposals to strengthen self-management organizations for the conservation of native birds in La Cuchilla, Nopala de Villagrán, Hidalgo, Mexico.

Organizational elements	Proposal
Criteria for the consolidation of the interest group	To identify peasant women who: • Have native hens in their production unit. • Are interested in the use, reproduction, and sale of native birds • Value the benefits, both tangible and intangible, that they can obtain from native hens.
Recognition of the appropriators' local lore	To identify, determine, and complement the knowledge that peasant women have regarding: • Phenotypic characteristics that distinguish native birds from "improved" birds. • The use of alternative incubation mechanisms to improve the number of chicks born (turkey hens, broody hens, among others). • Time of the year in which more birds are born (March-July). • Knowledge of the natural behavior of the native hen (brooding, feeding type, egg laying age, molting period). • Local forms of reproduction • Climatological characteristics for the best reproduction of birds • Indicators of success The aforementioned lore is little known by technicians or professionals, because they no longer work with these birds on a large scale.
Identification, recognition, and strengthening of binding contracts (local arrangements, system of rules)	• Definition of chick care and production practices. • Definition of the number of eggs to collect, incubate, and distribute. • Vertical-type organizations (a group that breeds them, a group that reproduces them, a group that sells them) with the possibility of rotating activities. • Mechanisms for the transmission of knowledge. • Dissemination of information. • Definition of the criteria that determine who can have access to the birds. • Definition of cooperation forms for the collective purchase of food, vaccines, and equipment that also lower the costs of these activities.

their potential and consequently take them into consideration and recognize their own organizations and their rights.

CONCLUSIONS

The participation of the community is essential for the *in situ* conservation of various zoogenetic resources. Nevertheless, the actions aimed at achieving it hardly consider the self-management organization that the community has or can develop as an effective way of conservation. The information obtained in this study shows the self-organization strategies that peasant women use to favor the conservation of their native hens, to which they attach great value due to their resistance to diseases and their adaptation to local feeding and environmental conditions. This situation helps to identify self-management as a way to conserve resources. Further studies about the self-management forms that women in rural communities use to conserve their native poultry resources will enable a more complex description of their functionality, potential, and cooperation mechanisms, among other elements that strengthen their structure.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Use of Near-Infrared Spectroscopy to Estimate Fiber and Crude Protein Content in Fodders

Sánchez-Valdés, Jair J.[1]; **Colín-Navarro, Vianey**[1]; **Castelán-Ortega, Octavio A.**[2]; **Rayas-Amor, Adolfo A.**[3]; **Rojo-Rubio, Rolando**[4]; **Estrada-Flores, Julieta G.**[1*]

[1] Instituto de Ciencias Agropecuarias y Rurales de la Universidad Autónoma del Estado de México. Campus UAEM El Cerrillo. El Cerrillo Piedras Blancas, Toluca, Estado de México, México. C.P. 50090.
[2] Facultad de Medicina Veterinaria y Zootecnia de la Universidad Autónoma del Estado de México. Campus UAEM El Cerrillo. El Cerrillo Piedras Blancas, Toluca, Estado de México, México. C.P. 50090.
[3] Universidad Autónoma Metropolitana Unidad Lerma. Departamento de Ciencias de la Alimentación. Lerma de Villada, Estado de México, México. C.P. 52006.
[4] Centro Universitario UAEM Temascaltepec, Temascaltepec, Estado de México, México C.P. 51300.
* Correspondence: jgestradaf@uaemex.mx

ABSTRACT

Objective: Demonstrate the need to use locally generated data in the calibration of a near-infrared spectrometer (NIRS) to predict the chemical characteristics of fodder; instead of using data bases from other geographic regions, as is commonly done in Mexico.

Design/Methodology/Approach: Two groups of samples collected in prairies of the central highlands of Mexico, the first group was used to calibrate the equipment; the equations generated were validated with a second group, collected in prairies that were different from the ones of the calibration group, but in the same geographic zone.

Results: The best regression coefficients of the NIRS predictions, compared to traditional laboratory analyses were for crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), dry matter (DM) and organic matter (OM) (0.93, 0.87, 0.87, 0.56, 0.72 y 0.68 respectively). The lowest predictive value was observed in ashes (0.27).

Limitations of the study/implications: The results show the need to use local materials in the calibration process.

Conclusions: NIRS will make predictions of their chemical composition, since this is influenced by geographic origin of the sample and its botanical composition.

Keywords: NIRS, chemical composition, native prairies, fiber, crude protein.

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INTRODUCTION

Ruminant production systems are based on fodder feed, predominantly on grazing on grasslands that are mainly native and non-cultivated. Livestock production on native and introduced grasslands depends on the amount of available fodder, expressed by its digestibility coefficient, its concentration of metabolizable energy and its crude protein (CP) content, which is conditioned by the type of species that form it. This in turn depends on the stage of the ecological succession that is present, the fertility level of the soil, amount, and distribution of rainfall, as well as the time at which it is used and the degree of intensity of this use (Villalobos *et al.*, 2000). Knowledge of these factors allows for optimal quality in the diet of ruminants that are fed with grasses, to achieve maximum productive response by the animal (Valente *et al.*, 2000).

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The analysis of the nutritional value of fodder normally includes the determination of its chemical composition and digestibility and is done with conventional laboratory methods. These are costly, take a long time to do and may be dangerous for people and the environment due to the great number of chemical products that are used (acid and alkaline substances) (Starks *et al.*, 2006).

In this sense, starting in 1970, the use of NIRS has been evaluated to determine the chemical composition of foods and fodder. This is instead of conventional techniques of wet chemistry, a technique which, when compared with conventional procedures, provides quick and reliable estimates, and does not pollute the environment since it does not use chemicals reactives (Starks *et al.*, 2006). NIRS is based on chemometrics, that is, on the application of mathematics to analytical chemistry, combines spectroscopy, statistics and computer science and generates mathematical models that relate the chemical composition (presence of active chemical groups) to changes in energy in the region corresponding to the near infrared (wavelengths between 800.0 and 2500.0 nm) (Ferret, 2003).

In the case of Mexico, most spectrometers contain data bases with calibration as well as validation spectra that were not generated in the country for the type of fodder and plants that are found here. For this reason, it is possible that their predictions are not sufficiently exact, since they do not consider differences between species, as well as climate and the agronomical management to which they are subjected, and which evidently have an effect on their chemical composition and nutritional quality. In several reports using NIRS technology, a common mistake made when estimating the chemical and nutritional value of fodder is to use equations that are derived for certain foraging species and using them to predict nutritional parameters in different species, which leads to inexact NIRS estimates (Beever and Mould, 2000).

Therefore, the objective of this work was to calibrate a near-infrared spectrometer in order to estimate crude protein content and fractions of fiber in samples of native grasses, introduced grasses and cultivated fodder from the highlands of central Mexico.

MATERIALS AND METHODS

Study site

The experiment was carried out in the state of Mexico (20° 17' and 18° 22' N and from 98° 36' to 100° 37' W), with an altitude above sea level of 2600 m. The climate is subhumid temperate (Cw) with summer rains and a mean temperature between 10.0 and 16.0 °C, with average rainfall of 876.2 mm (García, 2004). 2230 samples of grasses and fodder were collected from May, 2006 to December, 2007 (in the following municipalities: Almoloya de Juárez, Toluca, Temoaya, Rayón, Lerma and Mexicaltzingo), in four native prairies, two prairies with introduced grasses, two prairies with cut pasture and cultivated corn (*Zea mays*), barley (*Hordeum vulgare*), oats (*Avena sativa*), triticale (*Triticum* ssp.) and canola (*Brassica napus*); all of these are used by producers as feed for cattle.

Collection of samples

The sampling was divided into 18 periods of 30 days each. The method used was the one described by Hodgson (1994), using two metal frames, one measuring 0.25×0.25 m, specifically used to obtain samples in fodder crops, and another measuring 0.5×1.0 m, which was used in the prairies. The sampling site was randomly selected, tossing the metal frame into the air. Later on, the fodder in the frame was cut with grass shears, near the ground. While samples were collected, some species were taken to be identified in the laboratory. During each sampling period, 30 samples were collected in the native grasslands, 20 in the introduced ones and 10 in the crops.

Chemical analysis

In the laboratory, the samples were dried at 60.0 °C in the forced air stove until a constant weight was reached. Later on, they were ground and sifted with a screen that had a diameter of 1.0 mm. To do the wet chemistry analysis, samples were mixed for each collection period. Each sample was composed of 10 samples, obtaining a total of 223 compound samples. This was done in order to save on reagents. Like this, for each compound sample, content of DM, OM, CP and ashes (ASH) was determined, according to the AOAC (1990) methodology. NDF and ADF content was determined using the ANKOM method and the Van Soest *et al.* (1991) technique, and ADL content also was determined using the technique described by Van Soest and Wine (1967).

Calibrating the Near-Infrared Spectrometer

Using the 223 samples that were analyzed by wet chemistry, a spectrum was obtained in the near-infrared for which, from each compound sample, 10.0 grams were weighed in 50.0 ml beakers and brought to constant weight in a forced air stove at 60.0 °C. The samples were scanned in triplicate, using a Buchi NIR Flex N400 (Büchi) spectrometer, with Pb detector test tube, in the 1000 to 2500 nm reflectance wavelength range, generating a total of 669 spectra. Using NIRCAL software, version 4.01 (Büchi), a data base was created at the time when each sample's spectrum was obtained, relating each spectrum to the results from the wet chemistry analyses for each sample. Once the data base was created with the samples' spectra, these were divided into two groups or sets; two thirds of the spectra formed the calibration set (C-Set) and a third of the spectra formed the validation set (V-Set). To diminish the effects caused by particle size and dust, on the wavelength of the obtained spectra, three mathematical treatments were previously applied, which were the second derivative, standard normal variable, and the Kubelka-Munk treatment (Macho, 2002).

The method that was used to develop the calibration equations and for quantitative validation, was the partial least squares statistical method (PLS). The calibration equations were selected according to the least standard error prediction (SEP), the greatest correlation coefficient of each one of the sets (r^2 C-Set, r^2 V-Set) and the consistency of the equation (NIRVIS, 2000).

Validation of the Near-Infrared Spectrometer Predictions

To test the confidence level of the obtained calibration equations, an external validation was carried out using 30 samples that were collected at the Almoloya de Juarez municipality.

The samples were collected in 2006, at prairies with introduced grasses, which were different from those used to calibrate the equipment.

Analysis of Results

The values obtained in the validation were compared to the value obtained in the laboratory through a simple linear regression analysis and a Pearson correlation (Minitab, 2003). The percentage difference was also calculated (% Diff) between values obtained in the laboratory and the NIRS estimates, as reported by Stolter *et al*. (2006) in their study.

RESULTS AND DISCUSSION

Chemical composition

Table 1 shows the average, the interval of maximum and minimum values and the standard deviation for ASH, DM, OM, NDF, ADF, ADL and CP content of the samples used for calibration of the spectrometer. The variation that is observed in these chemical parameters is considered normal and may be attributed to the botanical variety of the collected samples, which were represented by one or more of the following species: *Pennisetum clandestinum*, *Sporobolus indicus*, *Juncus drummondii*, *Trifolium amabile*, *Paspalum paspaloides*, *Eleocharis dombeyana*, *Trifolium repens*, *Lolium perenne*, *Dactilis glomerata*, *Festuca arundinacea*, *Lolium multiflorum*, *Triticale* sp., *Vicia sativa*, *Brassica napus*, *Medicago sativa*, *Hordeum vulgare*, *Avena sativa*, *Zea mays*, among others.

The chemical quality is lower than that of cultivated fodder, Labaran *et al*., (2019) report values from 262.1 to 346.9 g kg^{-1} of MS, 97.6 to 262 g kg^{-1} ash, 734 to 902 g kg^{-1} MO, 121 to 162 g kg^{-1} MS for CP, 473 to 572 g kg^{-1}MS in FDN and 247 to 474 g kg^{-1} MS for FDA. But similar to tropical fodder, López-González *et al*. (2015) report values of 108 to 112 g kg^{-1} MS for CP, 606 to 658 g kg^{-1} MS of FDN and 378 to 399 g kg^{-1} MS in FDA content.

Calibration and validation of the Near-Infrared Spectrometer

The Table 2, shows the results obtained from the calibration, for each measured parameter, as well as the correlation coefficient for C-set and V-set, standard error prediction, the consistency of the equations generated for the internal comparison.

Table 1. Chemical composition expressed in g kg^{-1} of dry matter of samples used in the calibration of the spectrometer, showing number of samples (n), mean and range of values and standard deviation.

Parameter	n	Mean	Maximum	Minimum	Standard deviation
DM	223	932.2	987.8	882.1	18.1
OM	223	892.7	959.0	744.1	35.6
ASH	223	90.9	145.0	52.2	20.4
NDF	223	625.5	859.8	311.9	110.1
ADF	223	294.4	472.5	116.8	52.7
ADL	223	37.8	93.5	14.8	16.1
CP	223	115.0	289.0	30.1	55.6

NDF: Neutral detergent fiber; ADF: Acid detergent fiber; ADL: Acid detergent lignin; DM: Dry matter; OM: Organic matter; ASH: ashes; CP: Crude protein

Table 2. Correlation coefficients, standard error prediction and consistency of the equations generated.

Parameter	r^2 C-SET	r^2 V-SET	SEP	Consistency of the equation generated
DM	0.77	0.93	9.61	105.15
OM	0.80	0.77	12.40	101.68
ASH	0.76	0.76	13.59	101.31
NDF	0.90	0.87	48.68	101.56
ADF	0.90	0.89	25.23	99.07
ADL	0.78	0.77	10.02	125.90
CP	0.94	0.96	18.60	101.11

DM: dry matter; OM: organic matter; ASH: ashes; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; CP: crude protein; r^2 C-Set: correlation coefficient of the calibration set; r^2 V-Set: Correlation coefficient of the validation set; SEP: standard error prediction.

The models generated for the chemical parameters that were analyzed showed calibration and validation coefficients going from 0.76 for ASH, up to 0.96 for CP.

The SEP is a reliable quality indicator for the developed equation, since unlike SEC, which improves (values near zero) as new terms are added to the equation, SEP improves only until it starts to produce an over-adjustment of the equation, increasing (values far from zero) later on, with each new term (Alomar and Fuchslocher, 1998). The SEP of this work fluctuated between 9.61 and 48.68 for DM and NDF, respectively. Sapienza *et al.* (2008) state that the SEP are directly related to the standard deviation of the reference methods, that is, higher SEP values will be obtained when the standard deviation of the reference methods is higher; this is correct because the highest SEP was obtained in NDF (48.68) and its standard deviation from the reference method (Table 1) was 110.1. Along these same lines, Alomar and Fuchslocher (1998), mention that the calibration equations tend to have a better predictive value when they are developed on samples that are relatively homogenous, than when calibrations are done for more heterogenous populations, such as the ones in this work, where precision and accuracy tend to decrease.

With respect to the consistency of the equations that were generated, the obtained values were generally near 100. NIRVIS (2000) indicates that for a calibration to be acceptable, the consistency value must be close to 100, since it describes the relationship between the standard errors of the calibration and validation sets. In the case of the ADL fraction, we observe that it has a value of 125.90 for the consistency of the equation, a SEP of 10.02, and r^2 C-Set of 0.77 and an r^2 V-Set of 0.78. The SEP and consistency of the equation indicate that, in spite of having regression coefficients that were relatively low, the predictions that were made can be reliable.

Validation of the near-infrared spectrometer predictions

Table 3 shows the comparison between the laboratory values and the NIRS estimates, using samples that were completely different from those used for the calibration and from the sampling sites. The CP parameter got a regression coefficient of 0.93, slope of the

Table 3. Relationship between NIRS and the laboratory for the evaluated parameters, showing the regression equation, regression coefficient, % Diff and Pearson correlation coefficient.

Parameter	Regression equation	r^2	%Diff	R^2
DM	y=−0.3206x+1239.5	0.72	2.53	−0.85
OM	y=0.4739x+495.38	0.68	6.14	0.83
ASH	y=0.2488x+67.947	0.28	28.68	0.52
NDF	y=0.7424x+153.85	0.88	2.42	0.94
ADF	y=0.5435x+136.88	0.87	3.91	0.93
ADL	y=0.5689x+22.568	0.56	2.08	0.75
CP	y=0.9892x−13.354	0.93	10.69	0.96

DM: dry matter; OM: organic matter; ASH: ashes; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; CP: crude protein; r^2: regression coefficient; % Diff: percentage difference between laboratory values and NIRS values and R^2: Pearson correlation coefficient.

straight line of 0.99 and a mean difference between NIRS and the laboratory of 10.69%. The Pearson correlation coefficient (0.96) shows that the correlation is strong and the regression analysis confirms this by finding that the estimates made by NIRS were significantly related ($P<0.01$) to the reference analyses.

The results show that the NIRS technique may better predict the CP fraction in the case of the evaluated fodder species, in view of the highest observed regression coefficient, which was 0.93, Berardo (1997), obtained coefficients of 0.97 in white clover, García-Ciudad *et al.* (1999) got values going from 0.90 to 0.96 in heterogenous samples from native prairies in Spain, and Cozzolino *et al.* (2006) report coefficients of 0.90 for corn silage samples. These results are within the range of what is reported in the present study.

The NDF fraction got a regression coefficient of 0.87, a slope of 0.74 and a difference of 13 grams between the NIRS and the laboratory (2.42%). The analysis shows that the NIRS estimates are significantly related ($P<0.01$) to the laboratory analyses; in turn, the correlation coefficient (0.94) indicates that the correlation between NIRS and the laboratory is very strong.

For ADF, a difference of 3.91 % was obtained, a slope of the straight line of 0.54 and a regression coefficient of 0.87. From the regression analysis we have that NIRS estimates are significantly related ($P<0.01$) to the laboratory analysis. The Pearson correlation coefficient confirms this since there is a strong correlation between the NIRS and the laboratory.

In NDF and ADF contents, adequate coefficients were obtained of 0.87, which are within the range reported in the literature. Berardo (1997) found values of 0.97 and 0.96 for NDF and ADF respectively. Mentink *et al.* (2006) report 0.90 for NDF; Cozzolino *et al.* (2006) obtained coefficients of 0.84 for NDF and 0.81 for ADF in samples of corn silage.

For the DM content, a regression coefficient of 0.72 was obtained, a slope of the regression straight line of −0.32 and a difference between NIRS and the laboratory of 2.53%. The Pearson correlation coefficient (0.85) shows that there is a negative correlation between the laboratory and NIRS, which is confirmed by the P value indicating that the NIRS estimates were significantly related ($P<0.01$) to the reference analyses.

OM showed a 6.14% difference between NIRS and the laboratory, a 0.68 regression coefficient and the slope was 0.47. The P value ($P<0.01$) indicated that the reference analyses are significantly related to NIRS, which is confirmed by the Pearson correlation coefficient of 0.83.

For DM content, the regression coefficient was 0.72. This is greater than that reported by Garnsworthy *et al.* (2000) who obtained values of 0.47, which they attribute to the loss of humidity in the samples during storage. This inconvenience may be solved by determining DM content prior to the moment when the samples are explored; however, there are no published reports where the use of this procedure is mentioned in order to avoid low predictions. It is worth mentioning that the average weight of the samples that were used for the validation in this study was 931 g DM kg^{-1} and that in the spectrometer predictions, a mean was obtained of 956 g DM kg^{-1}, which explains the high regression coefficients observed in this work. Other authors state that NIRS is a technique that can be used to estimate DM, when finding coefficients greater than 0.80 (Cozzolino *et al.*, 2006). In any case, the prediction value that was obtained in this work may be considered to be acceptable.

The ADL content had a 0.56 regression coefficient and a 2.08% difference may be observed between NIRS and the laboratory, with a slope of the straight line of 0.57. The regression analysis shows that the NIRS estimates were significantly related ($P<0.01$) to the reference analysis and the correlation coefficient (0.75) indicates that the correlation is moderately strong.

The regression coefficient for ADL that was found in this work (0.56) was under that reported by Berardo (1997), who got a value of 0.89. This result may be explained as due to a great variation in the samples that were used to calibrate the equipment. Alomar and Fuchslocher (1998) state that the low determination coefficients in the fibrous fractions are probably due to the fact that the spectral data obtained through NIRS represent the real chemical structure of the sample, in a more precise manner than the determination done through wet chemistry, such as raw fiber, NDF, ADF, among others; these do not correspond to well defined molecular entities but to empirical determinations and thus, do not allow us to define the real chemical groups that are involved.

The ASH content had a 0.27 regression coefficient and a difference of 28.68% between NIRS and the laboratory; the slope was 0.25. The P value shows that NIRS estimates were significant ($P<0.05$) with the reference analysis. The Pearson correlation coefficient (0.52) shows that the relationship between NIRS and the laboratory was moderately strong.

Ashes are mainly composed of minerals, according to the principles of NIRS technique. This is based on the virtues of organic compounds, which are negatively related to inorganic compounds (Garnsworthy *et al.*, 2000). The regression coefficient for ASH was 0.27, a value that is under those reported in the literature. Mentink *et al.* (2006) got 0.77, Berardo (1997) 0.84 and Garnswothy *et al.* (2000), 0.93. However, in other works using compound feed (Murray, 1996) the low correlations for ashes are attributed to the variation in mineral content of feed, a situation which could be present in this work, given the heterogeneity of the samples that were composed of different grass species.

A calibration of the NIRS equipment is considered ideal if it predicts the chemical composition with an order of error that is similar to those achieved using wet chemistry; that is, that the calibrations depend on the analytical procedures that were used in order to provide reference values. The heterogeneity (of species, of the vegetative state, prairie and crop) of the samples used in this work, may have caused the relatively low regressions for ASH content. Alomar and Fuchslocher (1998) mention that the calibration equations tend to have a better predictive value when they are developed on samples with a relatively homogenous nature, or corresponding to the same type of product (*e.g.*, alfalfa hay). On the other hand, when one is trying to develop calibrations for more heterogenous populations with a broader base (*e.g.* straw, hay and silage from different plant species as a whole), precision and accuracy tend to decrease, as they did in this work, especially for ash content.

CONCLUSIONS

Considering the study conditions, we conclude that NIRS is a useful and economical tool that can be used to estimate some of the chemical characteristics (NDF, ADF, OM, CP, ADL and DM) of a broad spectrum of fodder samples. However, this technique proved to be less precise in the case of ASH estimates, for which further research is needed.

The drying of samples prior to obtaining their spectrum helps to improve the calibration of the spectrometer and hence its predictions. In order to maintain the structural and chemical integrity of the samples, the drying method that is mostly recommended is liofilization (Alomar and Fuchslocher, 1998).

It is necessary to calibrate the NIRS with locally obtained data, since as we have shown in this study, the variation coming from the diversity of species that are present in the region may affect the accuracy of the calibrations. For this reason, data bases of spectra that were obtained in latitudes other than those of Mexico, may not work for the fodder species that exist in the country.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Growth regulators in the rooting of sun poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch, Allg. Gartenzeitung) Valenciana variety

Rodríguez-Elizalde, María de los Angeles[1*]; Mejía-Muñoz, José Merced[1]; Espinosa-Flores, Amando[1]; Colinas-León, María Teresa[1]

[1] Departamento de Fitotecnia, Universidad Autónoma Chapingo. Km 38.5 Carr. México-Texcoco. Chapingo, Edo. de México, México, C. P. 56230.

* Correspondence: roelyma@gmail.com

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ABSTRACT

Objective: To improve the asexual propagation of the sun poinsettia (*Euphorbia pulcherrima*) Valenciana variety using growth regulators and monitoring the position of the buds on the stem.

Methodology: We compared root length, shoot length, and the number of roots, shoots, and leaves between control plants and plants with five different concentrations of three growth regulators (indole butyric acid, naphthaleneacetic acid, and gibberellic acid). We also evaluated the effect of the position of the axillary or vegetative buds on the stem on the same parameters. The data were analyzed by analysis of variance and multiple comparisons of means.

Results: The treatments with the highest root length, shoot length, and number of roots, nodes, and leaves were 2 A (AIB 200 mg L^{-1}, basal part), 3 A (AIB 300 mg L^{-1}, basal part), and 3 B (AIB mg L^{-1}, intermediate part).

Implications: This work helps solve a technical issue of sun poinsettia production in Mexico.

Conclusions: The application of indolbutyric acid at concentrations of 200 mg L^{-1} and 300 mg L^{-1} in stem cuttings of the intermediate and base promotes increased root and aerial development.

Keywords: auxins, ornamental plant, growth regulators.

INTRODUCCIÓN

The poinsettia (*Euphorbia pulcherrima*), also known as nochebuena, cuetlaxóchitl, Christmas flower, cardinal star, or federal star; is a plant native of Mexico, naturally distributed from Sinaloa to Guatemala. This species is cultivated worldwide thanks to the genetic improvement of specimens extracted from Mexico in 1828 by Robert Poinsett. The worldwide poinsettia market includes approximately 300 varieties of pot-planted poinsettias generated by greenhouse cultivation (Lack, 2011; Ecke *et al.*, 2004).

In Mexico, the poinsettia is commonly cultivated outdoors, growing bushily in orchards or family gardens and decorating with its colorful inflorescence. This species is a shrub with a height of up to 5 m and an inflorescence in the form of a cyathium, which consists of a female flower without petals

or sepals surrounded by individual male flowers (Ecke *et al.*, 2004). Nowadays, nine sun varieties are registered in Mexico's National Catalogue of Plant Varieties (CNVV) of the National Service for Seed Inspection and Certification (SNICS). Most of these varieties were obtained by horticulturists from the State of Morelos, Mexico, through the selection, breeding, and modification of creole poinsettia (García *et al.*, 2011; Colinas *et al.*, 2014) (Figure 1).

In particular, the Valenciana variety is distinguished by abundant bracts of different sizes, which form a kind of crest on the deformed red cyathia; this variety is the most commonly cultivated in Morelos. Horticulturists propagate it by cuttings, which are directly introduced into a substrate rich in organic matter without applying any hormonal treatment. This propagation procedure causes insufficient rooting, which, together with the scarce technical information regarding poinsettia cultivation, represents a problem for horticulturists.

Figure 1. Sun varieties registered in Mexico (A=Orejona, B= Juan Pablo, C=Valsu, D=Belén, E=Valenciana, F=Corona, G=Rehilete, H=Tete, I=Amanecer Navideño (Adapted from Colinas *et al.*, 2014).

The present work aims to offer an alternative for the cultivation of the Valenciana variety of poinsettia in Morelos. We used growth regulators and monitored the position of the buds on the stem to obtain more roots, thus improving the asexual propagation of the plant.

MATERIALS AND METHODS

Stems of sun poinsettia (*Euphorbia pulcherrima*) Valenciana variety were collected in March 2020 from the Germplasm Bank of the Universidad Autonoma Chapingo, Mexico (Figure 2), which is located at 19° 29’23” west and 98° 53’37” north.

The stems were cut into segments containing 2 to 3 buds of approximately 20 cm in length, taking care that the cut was clean, the stem was unbroken, and at least three buds were maintained after the cut. Once the semi-hardwood cuttings were obtained, they were grouped into three groups depending on the position of the cut in the stem (A=base, B=middle part, and C=upper or apical part of the stem), (Figure 3).

Aqueous solutions containing powdered reagent-grade growth regulators were prepared, dissolved with alcohol, and volumetrically diluted in water; the pH was adjusted to 5.7. The semi-hardwood cuttings were placed for 24 h in the solutions with different concentrations of the growth regulators. The cuttings maintained their polarity with respect to the base, and half of the cutting was placed in the solution. Once the time had elapsed, 12 cuttings from each treatment were placed in plastic boxes with a substrate containing peat moss+agrolite in a 2:1 v/v ratio. Half of the cutting was kept in the substrate to avoid dehydration during the rooting period.

The semi-hardwood cuttings were maintained in a glass greenhouse with temperatures ranging between 38 and 9 °C. The cuttings were irrigated two to three times per week

Figure 2. Poinsettia material in the germplasm bank of the UACh.

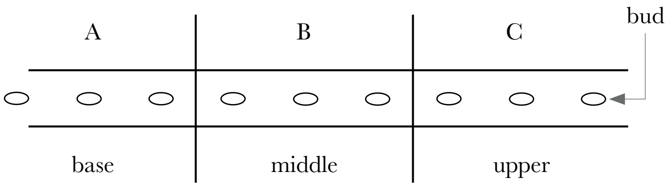

Figure 3. Semi-hardwood cutting according to stem position.

until evaluation three months later. Five treatments containing different concentrations of indolbutyric acid (IBA), naphthalenic acid (NAA), and gibberellic acid (GA_3) were evaluated along with a control. The treatments were as follows: 1) AIB 100 mg L^{-1}, 2) AIB 200 mg L^{-1}, 3) AIB 300 mg L^{-1}, 4) AIB 100 mg L^{-1}+ANA 100 mg L^{-1}, 5) AIB 100 mg L^{-1}+ANA 100 mg L^{-1}+AG_3 100 mg L^{-1}, and 6) no growth regulator (control). The parameters evaluated were root number, root length, node number, shoot length, and leaf number. The results were analyzed by analysis of variance; multiple comparisons of means were performed using Tukey's honest significant difference with significance level at 5%, and the general linear model theory was used with the GLM procedure of SAS (SAS Institute, 2015).

RESULTS AND DISCUSSION

After three months, the treatments 2 A (AIB 200 mg L^{-1}, basal part of the semi-hardwood cuttings) and 3 A (AIB 300 mg L^{-1}, basal part) generated the greatest amount of roots; while 5 A and 5 B (AIB 100 mg L^{-1}+ANA 100 mg L^{-1}+AG_3 100 mg L^{-1} in the basal and middle part, respectively) generated the least amount of roots (Table 1). These results suggest that the combination of AIB with other growth factors was not efficient for root generation; however, the application of AIB alone yielded a higher root production (Figure 4).

The application of auxin-based rooting agents promotes root production in poinsettia semi-hardwood cuttings. Since this species has a hollow stem structure, it is difficult to apply powdered rooting agents to the small contact surface; so the application in liquid form guarantees a better response. Similar results were found by Amri et al. (2010), who showed that the use of AIB promoted the rooting of *Dalbergia melanoxylon* (granadilla). Other ornamental species where AIB has been used successfully are *Hibiscus rosa-sinensis* L., *Conocarpus erectus*, *Rosa caninna* L., *Carisa carandas* L., *Streptosolen jamesonii*, and *Solidago*

Figure 4. Comparison of the worst treatment (5 B, 100 mg L^{-1}+ANA 100 mg L^{-1}+AG_3 100 mg L^{-1}, middle part) against the best one (3 A, AIB 200 mg L^{-1}, basal part) for rooting of *Euphorbia pulcherrima* Valenciana variety.

canadensis L. (Abdel-Rahman *et al.*, 2020; Tanuja and Rana, 2018; Pêgo *et al.*, 2019). AIB, a synthetic auxin, is commonly applied to various woody species for root generation because it regulates cell division, expansion, and differentiation (Frick and Strader, 2018). Commercial AIB is found alone or combined with other chemicals (Ludwig-Müller, 2000), making it easy to implement in horticulture; some examples of commercial AIB that could be used by horticulturists in Morelos include Radix®, Raizone®, Rootex®, among others.

Regarding the position of the buds, Table 1 shows that those located in the basal and middle part of the cutting generated a greater number of roots than those in the upper or apical part. These results are mainly because the basal and middle parts of the stems contain higher carbohydrate storage, a higher capacity for adventitious root formation, more organogenic activity, and higher micronutrient content (Zalesny *et al.*, 2003; Tsafouros *et al.*, 2019). Basal and mid-rooting has been reported in Rootpac-R and Myrobalan 29C compatible rootstocks in peach, nectarine, almond (Tsafouros *et al.*, 2019), sandalwood (Tate and Page, 2018), and granadilla (Amri *et al.*, 2010).

The treatments with the greatest root length were 2 A (AIB 200 mg L^{-1}, basal part) and 3 A (AIB 300 mg L^{-1}, basal part); while the lowest lengths were found in 5 A, 5 B, 5 C (AIB 100 mg L^{-1}+ANA 100 mg L^{-1}+AG_3 100 mg L^{-1} basal, middle and upper part, respectively) and 6 C (control, upper part) (Table 1).

Table 1. The effect of growth regulators on rooting and shoot development in semi-hardwood cuttings of sun poinsettia (*Euphorbia pulcherrima*) Valenciana variety.

Treatment		Number of roots	Root length (cm)	Number of buds	Number of leaves	Shoot length (cm)
1	A	18.08 abcd	12.50 abc	11.17 ab	14.00 a	57.75 a
	B	12.17 cd	6.17 abcd	9.25 ab	9.08 abcd	27.56 bcdefg
	C	5.50 d	5.71 abcd	8.67 ab	6.58 cde	14.54 fg
2	A	28.25 abc	13.58 a	13.75 a	11.33 abcd	46.25 abcd
	B	19.00 abcd	8.83 abcd	9.50 ab	9.83 abcd	31.25 abcdef
	C	5.83 d	4.42 dc	5.25 bcd	6.83 bcde	18.00 efg
3	A	34.33 a	13.13 ab	13.08 a	10.33 abcd	54.17 ab
	B	28.08 abc	12.88 abc	13.42 a	11.92 abc	48.83 abc
	C	7.25 d	8.08 abcd	8.33 abc	7.33 bcde	20.58 defg
4	A	32.25 ab	12.17 abc	5.42 bcd	7.08 bcde	24.75 cdefg
	B	14.25 bcd	6.46 abcd	2.58 cd	2.50 e	6.50 fg
	C	10.17 cd	4.79 bcd	2.17 d	1.83 e	4.00 g
5	A	3.58 d	2.94 d	1.83 d	5.50 de	8.83 fg
	B	1.58 d	1.96 d	1.58 d	2.75 e	3.92 g
	C	1.67 d	0.71 d	1.75 d	1.75 e	0.94 g
6	A	8.92 d	8.33 abcd	12.67 a	12.67 ab	44.17 abcdef
	B	6.92 d	7.90 abcd	11.83 a	12.25 abc	33.04 defg
	C	3.00 d	1.65 d	11.00 ab	8.92 abcd	20.58 defg
MSD		18.38	8.46	6.06	5.87	27.20

MSD=Minimum Significant Difference. Different letters in the same line denote significant differences (Tukey, α=0.05), n=12.

The results obtained in root length coincide with those obtained in root formation, again suggesting that the effectiveness of AIB is better without adding another growth regulator. AIB is known to promote root elongation and development in various crops (Frick and Strader, 2018); however, despite its use in agriculture since the 1950s, its effectiveness depends on the formulation, concentration, and species evaluated (Sisaro and Hagiwara, 2016). Ignoring the proper concentration of AIB can cause economic losses to producers (Ludwig Müller, 2000); therefore, the methodology proposed in the present experiment could increase the percentage of rooting and the length of roots generated in the cuttings.

The capacity of stem cell dedifferentiation and redifferentiation also determines root formation in different species (Amri *et al.*, 2010; Rout, 2006). In this sense, physiologically mature tissues have a lower rooting capacity than juvenile material (Aparicio *et al.*, 2014). In sun poinsettia variety Valenciana, the selection of stems of woody consistency and dark brown coloration generated few roots (pilot study, results not shown); therefore, we selected only those of semi-woody consistency and light brown color.

We also evaluated the development of the aerial part of the cuttings. The greatest shoot length was found in the 1 A treatment (AIB 100 mg L^{-1} basal part), while the 5 C treatment (AIB 100 mg L^{-1}+ANA 100 mg L^{-1}+AG_3 100 mg L^{-1} upper or apical part) gave the shortest length (Figure 5).

Regarding the number of nodes, the best treatments were 2 A (AIB 200-basal part), 3 A (AIB 300 basal part), and 3 B (AIB intermediate part); in contrast, the treatments with the lowest number of nodes were 5 B (100 mg L^{-1}+ANA 100 mg L^{-1}+AG_3 100 mg L^{-1} middle part) and 5 C (100 mg L^{-1}+ANA 100 mg L^{-1}+AG3 100 mg L^{-1} upper part) (Table 1).

The treatments with the highest number of leaves were 1 A (AIB 100 mg L^{-1} basal part), 3 A (AIB 300 mg L^{-1} basal part), and 3 B (AIB 300 mg L^{-1} middle part). These results can be explained by the position of the cuttings in the stem: thicker cuttings should have a higher concentration of cytokinins and more carbohydrates due to their proximity to the basal part (Oinam *et al.*, 2011; Rana and Sood, 2011). The development of the aerial

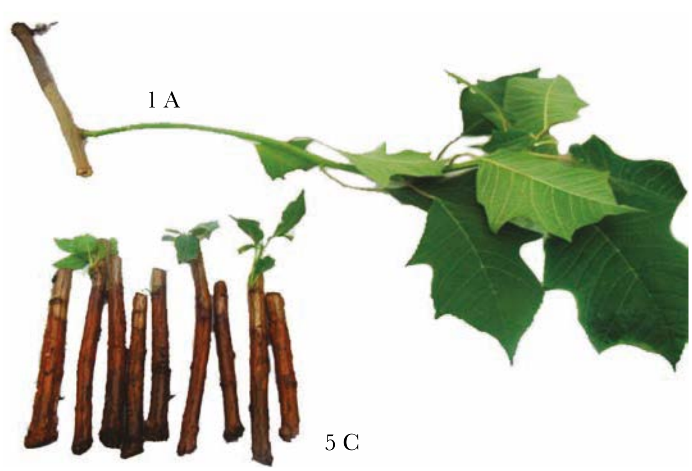

Figure 5. Shoot length in semi-hardwood cuttings of *Euphorbia pulcherrima* var. Valenciana in 1 A (AIB 100 mg L^{-1} basal part) and 5 C (AIB 100 mg L^{-1}+ANA 100 mg L^{-1}+AG_3 100 mg L^{-1} upper or apical part).

part, including the length of shoots and number of leaves, depends on two factors: first, the carbohydrate reserves contained in the stem, and, later, on the nutrients and water that are transported by the newly formed roots (Verwijst *et al.*, 2012). Thus, when the stems are cut, the upper part will stimulate the axillary buds, generating shoots. The shoots will first depend on the reserves of the cutting and, later, when the roots are formed, nutrients and water will feed them. Therefore, if the roots do not develop, the aerial part will die.

CONCLUSIONS

The treatments with the highest number of roots, nodes, and leaves, as well as greater root and shoot length, were 2 A (AIB 200 mg L^{-1}, basal part), 3 A (AIB 300 mg L^{-1}, basal part), and 3 B (AIB mg L^{-1}, intermediate part).

The growth regulator that gave the best development of roots and shoots was indol butyric acid (IBA) when applied to the base and intermediate part of the cuttings at the concentration of 200 mg L^{-1} and 300 mg L^{-1}.

The results obtained in the present experiment support that the application of IBA yields more effective rooting without the addition of other growth regulators. We recommend applying liquid IBA to the Valenciana variety poinsettia stems to improve current propagation methods.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Biostimulant effects of lanthanum (La) on crop growth, yield, and quality

Contreras-Martínez, Guadalupe[1]; **Gómez-Merino, Fernando C.**[2]; **Peralta-Sánchez, María G.**[1]; **Alcántar-González, Gabriel**[1]; **Trejo-Téllez, Libia I.**[1*]

[1] Colegio de Postgraduados Campus Montecillo. Montecillo, Texcoco, Estado de México, México. C. P. 56230.
[2] Colegio de Postgraduados Campus Córdoba. Manuel León, Amatlán de los Reyes, Veracruz, México. C. P. 94953.
* Correspondence: tlibia@colpos.mx

ABSTRACT

Objective: To analyze the various effects that the use of La as an inorganic biostimulant has on growth, yield, and quality of different crops.

Design/Methodology/Approach: We explored and analysed recent literature concerning the effects of lanthanum on various economically important crops. Subsequently, the most relevant information was selected, analyzed, and grouped by type of effect.

Results: The addition of low doses of lanthanum has been tested in different crops. La has been proven to increase growth, development, and quality in various species. Likewise, positive effects have been reported in germination, in the absorption of nutrients, and in the mitigation of the deficiencies of some essential elements, as well as in the promotion of physiological and biochemical responses.

Study Limitations/Implications: The analyzed results have been generated in a great diversity of plant species, under different production systems, and with dissimilar doses, as well as with different sources and application methods. This situation presents a challenge, since it hinders the possibility to issue general recommendations.

Findings/Conclusions: Lanthanum improves yield and quality, as well as some physiological, biochemical, and nutritional responses in different crops of economic importance.

Keywords: rare earth elements, hormesis, biostimulation.

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INTRODUCTION

Plant biostimulation consists of the application of some substances or microorganisms (Figure 1) to plants to promote an efficient nutrient use, increase stress tolerance, and improve plant quality, regardless of their nutritional content (Du Jardin, 2015).

Biostimulants can be of natural or synthetic origin and have an organic or inorganic composition. Natural biostimulants may include plant and seaweed extracts, while synthetic ones may be represented by beneficial elements and phosphite salts.

Among the inorganic compounds, the positive effects of phosphite and various elements of the so-called "beneficial" group —including mainly alkaline-

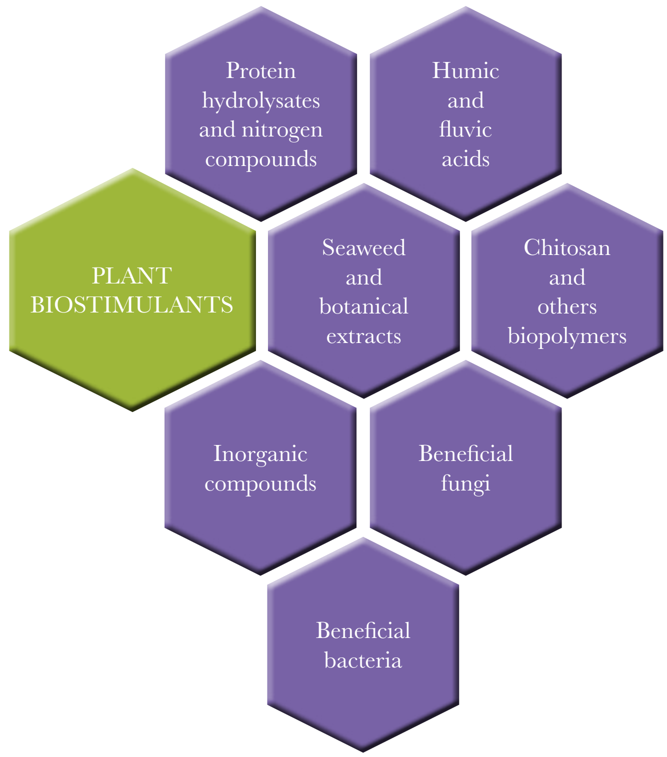

Figure 1. The current seven categories of biostimulants used in crop production.

earth elements, transition metals, and metalloids— have been recorded (Gómez-Merino and Trejo-Téllez, 2015). These elements are called beneficial because, provided in small amounts, they promote growth, quality, and yield in various crops (Ramírez-Martínez *et al.*, 2012). Consequently, they represent an alternative for the current agronomic problems, such as loss of fertile soils due to erosion, nutrient depletion, organic carbon loss, soil sealing, industrialization, and climate change (FAO, 2015). The beneficial transition metals include the rare earth elements (REE), a group to which lanthanum (La) belongs.

Rare earth elements in agriculture

Plants require at least 17 mineral elements (C, H, O, N, P, K, Ca, Mg, S, Cl, Fe, B, Mn, Zn, Cu, Mo, and Ni) to complete their life cycle; they are mainly acquired from the soil or are included in a nutrient solution. However, plants also accumulate small quantities of non-essential elements (Watanabe *et al.*, 2007). These elements include the REEs that, in low concentrations, can stimulate physiological and biochemical processes, growth, development, and yield in plants, improve the stress tolerance of the plant and the absorption of elements, and stimulate the resistance mechanisms of biotic and abiotic factors (Kastori *et al.*, 2010; Liu *et al.*, 2013; Gómez-Merino and Trejo-Téllez, 2018). REEs have certain effects on the activity of some enzymes, the content of phytohormones, the productivity and intensity of photosynthesis, the development of chloroplasts, chlorophyll content, resistance to water stress, the symbiotic fixation of atmospheric nitrogen, seed germination, and the growth and productivity of many crops (*e.g.*, rice, sugarcane, sugar beets, soybeans, and sunflowers). Interestingly, the Casparian strip of the root may lead to differences in the mobility of REEs —as the group of lanthanides that contains the element lanthanum (La)— limiting its transport in the root. Therefore, the absorption is much faster through the leaves than through the root (Hu *et al.*, 2004; Kastori *et al.*, 2010).

Lanthanum as an inorganic biostimulant in plants

Lanthanum (La) was isolated as an oxide: a cerium nitrate impurity contained in cerite minerals. It was discovered by the Swedish chemist Carl Gustaf Mosander in 1839, who gave it the name lanthanum. Its name derives from the Greek "lanthanein" (*i.e.*, "the hidden one"), a name given because its identification was a difficult process (González, 2019).

Small amounts of lanthanum have been applied to promote the growth and development of higher plants, taking advantage of hormesis, a phenomenon characterized by stimulation, when applied at low doses, and inhibition, when applied at high doses (Kobayashi *et al.*, 2007; Brioschi *et al.*, 2013; Calabrese and Blain, 2009; Agathokleous *et al.*, 2018). Table 1 shows some examples of studies carried out with different lanthanum sources in various plant species and the concentrations evaluated. In all cases, the doses are low (50-250 μM La) aiming at the zone of positive effects.

Table 1. Examples of lanthanum (La) doses evaluated in different species of model and cultivated plants.

Crop species	Source	Dosis		References
		Minimum	Maximum	
Barley (*Hordeum vulgare* cv. Clipper)	La^{+3}	10^{-2} M	10^{-4} M	Van Steveninck *et al.* (1976).
Rice (*Oryza sativa*)	$La(NO_3)_3$	100 $\mu g\ g^{-1}$	700 $\mu g\ g^{-1}$	Fashui *et al.* (2000).
Arabidopsis (*Arabidopsis thaliana*)	$La(NO_3)_3\ 6H_2O$	0.5 mM	50 mM	He and Loh (2000).
Tobacco (*Nicotiana tabacum* L.)	$LaCl_3$	5.0 mg L^{-1}	100 mg L^{-1}	Chen *et al.* (2001).
Wheat (*Triticum durum*)	$LaPO_4$	0.5 mg L^{-1}	25 mg L^{-1}	Hu *et al.* (2002).
Barley (*Hordeum vulgare* L. cv. YC 01301)	$La(NO_3)_3\ 6H_2O$	2 μM	100 μM	Han *et al.* (2005).
Lettuce (*Lactuca sativa* L.)	$La(NO_3)_3$	0.04 mg L^{-1}		He *et al.* (2005).
Arabidopsis (*Arabidopsis thaliana*)	$LaCl_3$	1.0 μM	1.5 μM	Kobayashi *et al.* (2007).
Wheat (*Triticum durum*)	$La(NO_3)_3$	0.01 mM	10 mM	D´Aquino (2009).
Tulip (*Tulipa gesneriana* L.)	$LaCl_3$ y $La(NO_3)_3\ 6H_2O$	0.5 μM	40 μM	Ramírez-Martínez *et al.* (2012).
Rice (*Oryza sativa*)	$La(NO_3)_3$	0.05 mM	1.5 mM	Liu *et al.* (2013).
Cucumber (*Cucumis sativus* L.)	La_2O_3	0.2 mg L^{-1}	2000 mg L^{-1}	Ma *et al.* (2015).
Soybean (*Glycine max* L. Merrill).	$La(NO_3)_3\ 6H_2O$	5 μM	160 μM	De Oliveira *et al.* (2015).
Pepper (*Capsicum annuum* L. cv. Sven, Sympathy, Yolo Wonder and Zidenka)	$LaCl_3$	10 μM		García-Jiménez *et al.* (2017).
Lisianthus (cv. Mariachi Blue and Echo Lavanda).	$La(NO_3)_3\ 6H_2O$ $LaCl_3$	10 μM	30 μM	Torres-Flores *et al.* (2018).
Pak choi (*Brassica chinensis* L.) and sunflower (*Helianthus annuus* L.)	La_2O_3	20 mg kg^{-1}	300 mg kg^{-1}	Rezaee *et al.* (2018).

The term hormesis means "to set in motion" or "to drive something forward". This natural phenomenon is a response to stress, which leads to an adaptive compensatory process against potentially toxic substances or compounds that affect organisms (Poschenrieder *et al.*, 2013).

The REEs effects have been researched in the various biological processes mediated by calcium in plants. As a Ca analog, La has been called "supercalcium" (Brown *et al.*, 1990). Therefore, Kastori *et al.* (2010) have proposed that the application of La^{+3} can mitigate Ca deficiency symptoms and can stimulate plant growth, as well as the stability, permeability, and functioning of cell membranes. Nevertheless, La inhibits many enzymes and other functional proteins. When it displaces Ca at extracellular binding sites, the extracellular and intracellular Ca efflux can be inhibited, which has negative consequences for the plant (Thomas *et al.*, 2014).

La has a positive influence on nutrient absorption, which may be due to the fact that it is the most electropositive of the REEs and its chemical properties are similar to alkaline-earth elements, including Ca and Mg. La has positive effects on K uptake in cotton (*Gossypium hirsutum* L.), wheat (*Triticum aestivum* L.), and rice (*Oryza sativa* L.). Furthermore, the application of lanthanum chloride increases the K^{+} and Mg^{+2}-ATPases activity of the cell membrane under Ca deficiency conditions (Ramírez-Martínez *et al.*, 2012).

The application of 5 and 20 μM of La to tulips increases the bioaccumulation of potassium, calcium, and lanthanum (Ramírez-Martínez *et al.*, 2012). In rice, low concentrations of La^{+3} improved root growth and increased the accumulation of K, Mg, Ca, Na, Fe, Mn, Zn, Cu, and Mo (Liu *et al.*, 2013). In sunflower (*Helianthus annuus* L.), treatment with low doses of La and Nd ($<$100 mg kg^{-1}) had positive effects (Rezaee *et al.*, 2018). However, high concentrations of La can affect the absorption of Ca, Fe, Cu, Zn, Mg, Mn, P, and K in faba bean (*Vicia faba* L.) seedlings (Liu *et al.*, 2012).

The positive effects on nutrient uptake can be reflected in plant growth. Such is the case of pepper (*Capsicum annuum* L.) seedlings: the treatment with La increased their height, stem diameter, number of flower buds, and leaves (García-Jiménez *et al.*, 2017).

La has also positive effects on the germination of old rice seeds (Fashui *et al.*, 2000), by promoting respiration and the activities of the superoxide dismutase, catalase, and peroxidase enzymes (Olivares *et al.*, 2011; Liu *et al.*, 2012). Therefore, it is suggested that La can reduce oxidative stress.

La favors chlorophyll content, improving growth, development, yield, and quality in crops (Turra *et al.*, 2015; Luo *et al.*, 2021). Its application has improved the quality of potted and postharvest flowers. In two varieties of lisianthus (*Eustoma grandiflorum* L.), doses of 10 μM of La increased the life of the potted flower (Torres-Flores *et al.*, 2018). During the vase life of the tulip, the application of La enlarged the length and diameter of the buds and the length of the stem, improving water absorption, which was reflected in a fresh weight increase of the flower stem (Gómez- Merino *et al.*, 2020a). During the final stage of the tulips' vase life, an increase in the concentration of chlorophyll was recorded (Gómez-Merino *et al.*, 2020b). Figure 2 shows a summary of the biostimulant effects of lanthanum on crop production.

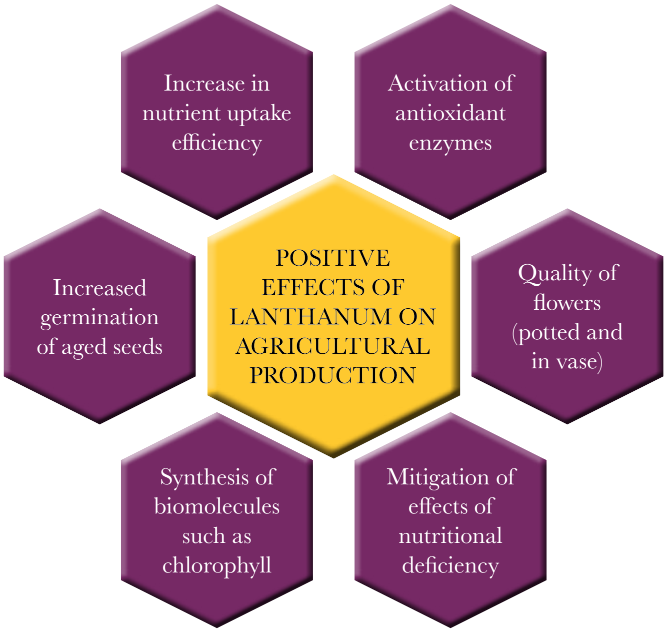

Figure 2. Some of the biostimulant effects of lanthanum (La) on crop production.

CONCLUSIONS

Lanthanum is a beneficial element that stimulates plant metabolism in a hormetic manner. Applied in adequate concentrations and considering hormetic limits, La can improve various processes such as nutrient absorption, activation of antioxidant enzymes, and improvement of photosynthetic indicators. This leads to the improvement of the growth, yield, and quality indicators of the crops, as well as to a greater capacity to face abiotic environmental stress.

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Effect of the larval density and food ration on *Penaeus vannamei* (Boone, 1931) zoea

López-Peraza Diana J.[1]**; Bermudes-Lizárraga, José F.**[1*]**; Flores-Alarcón Juan M.**[1]**; Nieves-Soto Mario**[1]**;**

[1] Universidad Autónoma de Sinaloa. Facultad de Ciencias del Mar. Mazatlán, Sinaloa, México. Paseo Claussen S/N. Centro. C. P. 82000

* Correspondence: bermudes@uas.edu.mx

ABSTRACT

Objective: To analyze the combined effect of food and larval density in order to determine the combination which the best results of the development, growth and survival of the *Penaeus vannamei* zoea are obtained.

Desing/methodology/approach: The experiment consists of evaluating 16 treatments resulting from the combination of four larval densities: 100, 200, 300 and 400 larvae L^{-1} and four densities of *C. muelleri* as food, adjusting the rations in each of the three phases of the larval development.

Results: The highest values of the development index (3.7), growth in terms of total length (2.86±0.09 mm) and dry (62.13±10.41 μg larvae^{-1}) and organic weight (50.83±7.51 μg larvae^{-1}), as well as the survival (88.30±9.10%) at the end of the experiment were obtained in treatment 3, which consisted of an intermediate larval density (300 larvae L^{-1}) and low food concentration.

Study limitation/implications: For a distintic species of microalga as food, it will be required to evalue the density to use.

Conclusions. In order to optimize the performance of shrimp larval cultivation, the use of a density of 300 larvae L^{-1} and rations of 50 to 80×103 cel mL^{-1} of *C. muelleri* are recommended for future experimental cultivation of *P. vannamei* zoea.

Keywords: *Chaetoceros muelleri*, development, growth, shrimp, survival.

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INTRODUCTION

Shrimp is a resource of great importance in Mexico, due to the high demand of the population for this resource, the trend in shrimp farming is the production of high biomass per unit area. However, one of the greatest challenges faced by this activity is the massive production of shrimp larvae and juveniles of and adequate nutritional quality, required by commercial producers (Pérez-Morales *et al.*, 2016). In this sense, the quantity and nutritional quality of the food supplied to the organisms is crucial, since, directly influences the growth, development and survival of the larvae (Martínez-Córdova *et al.*, 2014).

Image by Tuan Trieu Thanh at Pixabay

Various balanced feeds have been formulated for cultured organisms however, live food remains essential, at least in the critical phases of development (larvae and juveniles) of species of commercial interest (Sánchez-Estudillo, 2011). Live food has several advantages over balanced feed, including its high nutritional value, and greater availability and digestibility of nutrients (Castro *et al*., 2003).

Among the live food mostly used by shrimp larvae production, there are different species of microalgae belonging to the genera of *Chaetoceros*, *Skeletonema*, *Tetraselmis*, *Thalassiosira*, *Dunaliella* and *Isochrysis*, as well as zooplankton organisms such as the *Artemia*; the latter is suitable for feeding white shrimp mysis and postlarvae. However, laboratory feeding protocols are commonly based on personal or empirical experiences, which means that the feed supplied is not efficiently used (Aguirre-Hinojosa *et al*., 1999, Piña-Valdez *et al*., 2006). A high density of organisms in the cultivation can cause negative effects on its yield (Mena-Herrera *et al*., 2006; Li *et al*., 2007; Neal *et al*., 2010; Sookyng *et al*., 2011), associated, mainly, to competition for food and aggression between individuals (Sellars *et al*., 2004; Araneda *et al*., 2008; Krummenauer *et al*., 2010). It is essential to establish an adequate relationship between the density of larvae in the cultivation and the food ration to be supplied. In this sense, some studies have focused on the search for the density of postlarvae (Krummenauer *et al*., 2010; Lorenzo *et al*., 2016) and juveniles (Neal *et al*., 2010; Costa *et al*., 2016) of shrimp which a higher yield is obtained under different culture conditions. Efforts have also been made to determine the appropriate concentrations of microalgae (from 30 to 110×10^3 cel L^{-1}) as food for shrimp zoea, in culture densities ranging from 120 to 150 larvae L^{-1} (Kumlu, 1998; Valenzuela-Espinoza *et al*.,1999; Artiles-Rodríguez, 2000; Piña-Valdez *et al*., 2006; Costa *et al*., 2016). The highest survival (>80%) of the *Penaeus schmitti* zoea was obtained when they were fed with the microalgae *Chaetoceros gracislis* at a rate of 30 to 60×10^3 cel L^{-1} (Artiles-Rodríguez, 2000), while in *P. vannamei* (>51% survival) was recorded using the microalgae *C. muelleri* as food, adjusting the rations in each of the three substages of zoea of 100, 150 y 200×10^3 cel L^{-1}, respectively (Piña-Valdez *et al*., 2006).

Nevertheless, there is no standard feeding protocol established according to the stage and density of the larvae that guarantees a better use of the food and therefore a better crop yield. For these reasons, the main objective of this study was to determine the concentration of food (*C. muelleri*) and the density of the larvae which the best development, growth, and survival of the zoea larvae of *L. vannamei* are obtained.

MATERIAL AND METHODS

The *P. vannamei* larvae used in this study were donated by the company "Proveedora de Larvas S.A. de C.V." (FITMAR). The experiment began when 70% of the larvae were at nauplii V and ended on the fifth day, when 90% of the larvae in the treatments were at mysis I. The experiment consisted of evaluating 16 treatments resulting from the combination of four initial larval densities: 100, 200, 300 and 400 larvae L^{-1} and four food rations of *C. mulleri*, adjusting the rations in each of the three phases of development (Table 1). The concentrations added were from 50 to 200×10^3 cel mL^{-1} for zoea I, from 65 to 260×10^3 cel mL^{-1} for zoea II and from 80 to 320×10^3 cel mL^{-1} for zoea III.

Table 1. Treatments (T) resulted from the combination of four densities (D) of larvae and four rations of *Chaetoceros muelleri* microalgae as food (F) for *Penaeus vannamei* zoea.

	Food ration (10^3 cel mL^{-1})			Density (larvae L^{-1})			
	Zoea I	Zoea II	Zoea III	D1 100	D2 200	D3 300	D4 400
F1	50	65	80	T1	T2	T3	T4
F2	100	130	160	T5	T6	T7	T8
F3	150	195	240	T9	T10	T11	T12
F4	200	260	320	T13	T14	T15	T16

Each treatment was performed in quadruplicate. In total, 64 circular experimental units with transparent walls of 12 L capacity were used, which were randomly distributed on four wooden shelves of three levels each. The temperature in the treatments was maintained at 30 °C with the help of a 50 W titanium heater (Finnex model HMT50), 35 ups of salinity and continuous aeration. It should be noted that the experiment was repeated twice, therefore, the data presented in this work represent the mean of 8 replicates, four per experiment.

Production of the microalgae *Chaetoceros muelleri*. For the cultivation of the microalgae, seawater filtered to 1 μm and passed through a filter with an activated carbon cartridge was used. The filtered water was stored in 200 L containers where 5% sodium hypochlorite was added at a rate of 1 mL L^{-1} for sterilization at least for least 24 h. At the time of use, residual chlorine was removed by adding sodium thiosulfate at a rate of 60 mg L^{-1}. The F medium (Guillard and Rhyther, 1962) for cultures was used.

The cultures were carried out in transparent polyethylene terephthalate containers of 16 L capacity each one. The cultures were made in series, harvesting daily at 48 h. They were kept with constant aeration and a light intensity of 6000-6500 lux. Cell density was estimated by counting in a compound microscope (Olympus model CH30), using a Neubauer haemocytometer camber.

Analysis of the samples. During the experimental cultures of the larvae, the development index, growth in terms of total length and in dry and organic weight, as well as survival, were estimated.

Larval development index. 30 larvae were taken from each of the experimental units at intervals of 6 h, which were observed directly under a stereoscopic microscope (Leica model Zoom 2000) to identified the development phase. Then, they were immediately returned to their respective treatments, thus minimizing sampling mortality. Subsequently, the larval development index (ID) was determined using the following equation proposed by Villegas and Kanazawa (1979):

$$ID = \frac{\sum in_i}{n}$$

i is the absolute value attributed to each larval stage (nauplii V=0, zoea I=1, zoea II=2, zoea III=3, mysis I=4, mysis II=5, mysis III=6, PL1=7). ni is the number of organisms of the corresponding phase of value *i* found in a sample, and *n* is the total number of specimens observed in each sample.

Growth. The total length of the larvae was measured every 24 h; for this, 30 larvae per treatment were randomly selected and placed in the fixative solution described by Correa Sandoval and Bückle Ramírez (1993). Subsequently, the larvae were measured using a compound microscope (Olympus model CH30), equipped with an eyepiece reticle.

To determine the initial and final dry and organic weight of the larvae (n=300), they were concentrated on Whatman GF/C glass fiber filters of 25 mm diameter previously calibrated at constant weight. After the residual salt was eliminated with 4% ammonium formate, the filters were placed at a temperature of 60°C for drying and weighing. Once the constant dry weight of the samples was obtained, they were incinerated at 250 °C for 4 h, to obtain the ash weight of the larvae. The organic or ash-free weight of the larvae was calculated by the difference between the dry weight and the inorganic or ash-free dry weight of the larvae.

Survival. was determined every 24 h by direct counts of live larvae, in 500 mL samples from each experimental unit. It is worth mentioning that, daily, a 30% seawater exchange was carried out in the experimental units, and based on the estimation of the survival of the larvae, the volume of the treatments was adjusted to keep the initial density of the organisms as a constant, and thus avoid density-dependent responses.

Statistical analysis. After verifying the normality (Lilliefors test) and the homogeneity of variance (Bartlett test) to the data: development index, total length, and survival of the shrimp zoea, a two-way analysis of variance (ANOVA) was applied to determine the significant differences between treatments. When the ANOVA revealed significant differences, Tukey's multiple comparison test was applied to identify these differences (Figures 1, 2, 3 and 5). The results are expressed as mean ± standard deviation (n=8). Symbol * and different letters indicate significant differences between treatments. All tests were performed with a significance level of (α) of 0.05 (Zar, 2010).

RESULTS AND DISCUSSION

Development index. The development index of shrimp larvae showed a similar trend until 90 h (Figure 1), where a total average of 2.98±0.03 was recorded, which indicated that 98±0.03% of the larvae in the experiment were at the zoea III stage. The highest values (3.97) of the DI at the end of the experiment were recorded at T1 and T3, but they only showed significant differences with the values recorded at T9 and T13 (Figure 1). The maximum DI obtained was higher than the maximum value (DI=3.17) recorded by Piña-Valdez *et al.* (2005) feeding with the same microalgae (*C. muelleri*), and same period of time. But the studies carried out by Medina-Jasso *et al.* (2004) and Bermudes-Lizárraga (2009) recorded an accelerated development of *L. vannamei* zoea feeding monogal diets of *C. muelleri* and *Thalssiosira weissflogii*, respectively. These authors recorded similar values of DI (above 95% of larvae in mysis I) in a shorter period of time; at 72 and 102 h, that is, up to 48 h earlier than in the present study. Although Medina-Jasso *et al.* (2004) recorded an advance in DI, the total length of the larvae was similar to that obtained in this study.

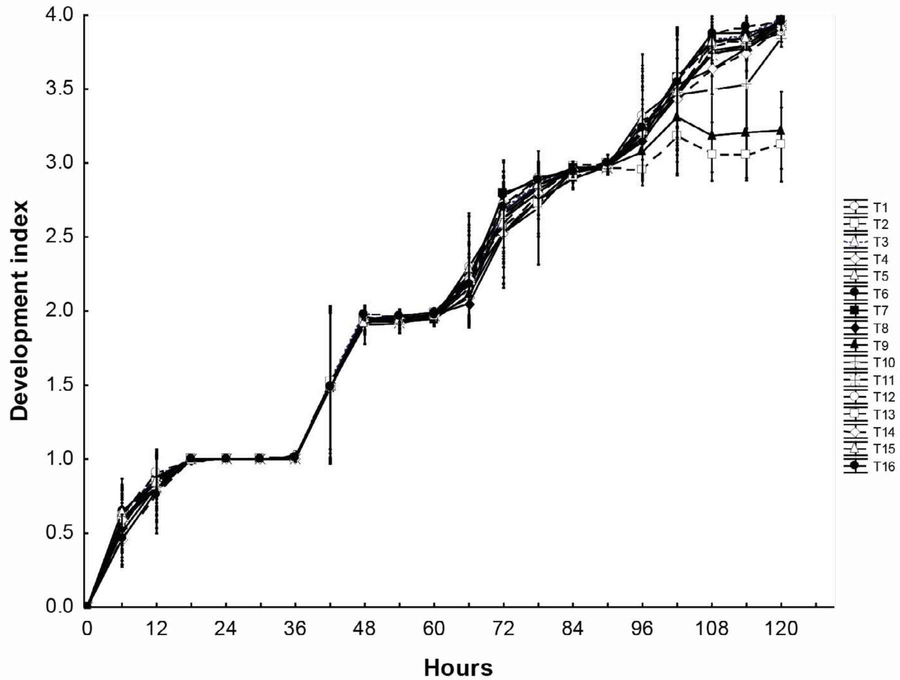

Figure 1. Development index of *P. vannamei* zoea fed with *C. muelleri* microalgae.

Likewise, the DI registered at 120 h was similar to that registered in zoea of *L. stilyrostris* fed with a mixture of *C. calcitrans*, *T. suecica* and *Artemia nauplii* (Isiordia-Pérez and Puello-Cruz, 2007). This suggests that the zoea of *L. stylirostris*, like those of *P. vannamei*, could be fed only with phytoplankton (Kumlu et al., 1998; Valenzuela-Espinoza, 1999; Piña *et al.*, 2005, 2006; Bermudes-Liárraga, 2009), with not need to add zooplankton organisms such as *Artemia*, which would imply a reduction in feeding costs.

Growth. There were no significant differences between the total length of the larvae of the treatments until day 3, however, at day 4, T13 was significantly lower than the rest of the treatments, which did not present differences among them (Figure 2). At day 5, the highest average of the total length recorded was 2.86±0.09 mm in T3, which represented an increase by 6.6 times compared to the initial value of the experiment (0.43 mm), however, this result was only significantly different from the values presented at T9 (2.42±0.30 mm) and T13 (2.40±0.14 mm).

The average unit dry weight at the beginning of the experiment was 2.59±0.20 μg larvae^{-1}, the organic weight was 2.31±0.05 μg larvae^{-1} and the percentage of organic matter with respect to dry weight (OW/DW) was 89.55±5.25%. The highest unit dry weight at the end of the experiment was 62.13±10.41 μg larvae^{-1} in T3, which indicates that the larvae increased their size about 24 times, compared to the initial value. This unit dry weight value was statistically different only from what was recorded at T9 and T13, which did not present differences among them (Figure 3). Similarly, T3 recorded the highest unit organic weight (50.83±7.51 μg larvae^{-1}) at the end of the experiment (22 times more in relation to the initial value), being significantly different from the values recorded in treatments 1, 5, 9, 10, 13 and 14. Likewise, the maximum unit dry weight obtained in this work is almost double that recorded by Piña-Valdez *et al.* (2005).

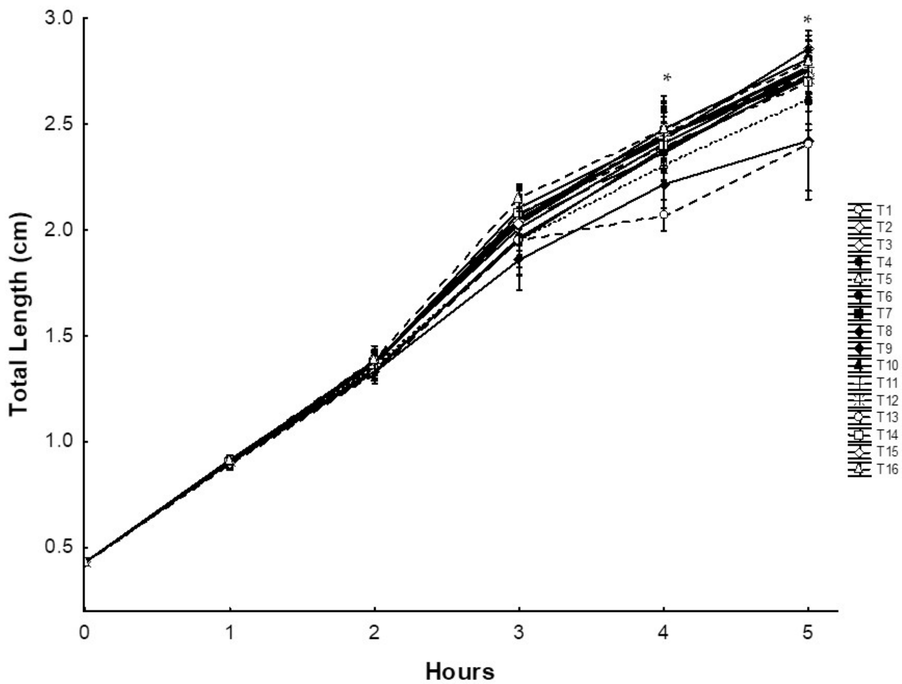

Figure 2. Total length (mm) average *P. vannamei* zoea fed with *C. muelleri* microalgae.

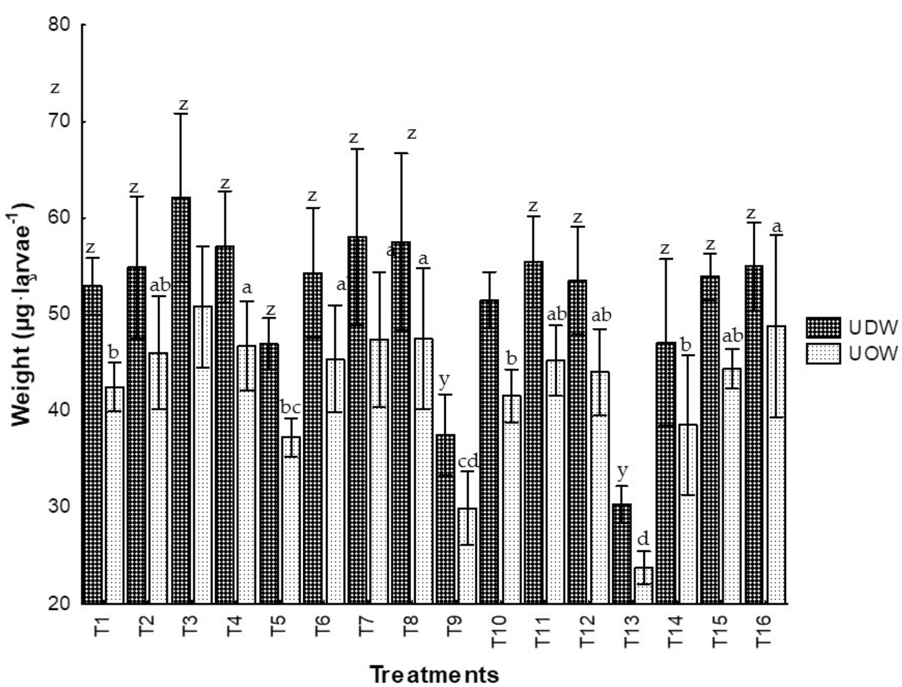

Figure 3. Final unit dry weight (UDW) and organic weight (UOW) of *P. vannamei* larvae fed with *C. muelleri* microalgae.

In relation to the final OW/DW content, the values fluctuated between 78.45±2.80 and 84.04±4.52% (T13 and T2, respectively), without significant differences between treatments (Figure 4).

Survival. In the same way as in the total length, the differences between the percentage of survival among the treatments were recorded from the fourth day of culture (Figure 5). On this day, T3 recorded the highest survival (88.30±9.10%), however, it only presented significant differences with T1, T4, T5, T7 and T11, which were not differences among them. At the end of the experiment, T3 registered a final survival of 87.67±7.11%, being significantly higher than the rest of the treatments. The maximum final survival of this study was higher than the values recorded in previous studies (Medina-Jasso *et al.*, 2004; Piña-Valdez *et al.*, 2005, 2006; Isiordia-Pérez *et al.*, 2007) for *P. vannamei* zoea, but it was similar to those obtained for *P. indicus* fed with mixed diets of *Tetraselmis chuii* and *Skeletonema costatum* (Kumlu, 1998), *L. vannamei* fed with monoalgal diets of *C. muelleri* (Valenzuela-Espinoza *et al.*, 1999), *L. schmitti* fed with *C. gracilis* (Godínez *et al.*, 2005) and for *L. stylirrostris* fed with *C. calcitrans* (Godínez *et al.*, 2005), which survival values from 87 to 92.7% were recorded.

As in other studies (Kumlu, 1998; Bermudes-Lizárraga, 2009), the increase in the amount of food supplied to the *P. vannamei* zoea did not reflect a proportional increase in growth in terms of total length and dry and organic weight, nor in the DI. On the contrary, treatments 9 and 13, which had a low larval density (100 larvae L^{-1}) and a high amount of food, particularly T13 (D100C4), were the ones that registered the lower values of these

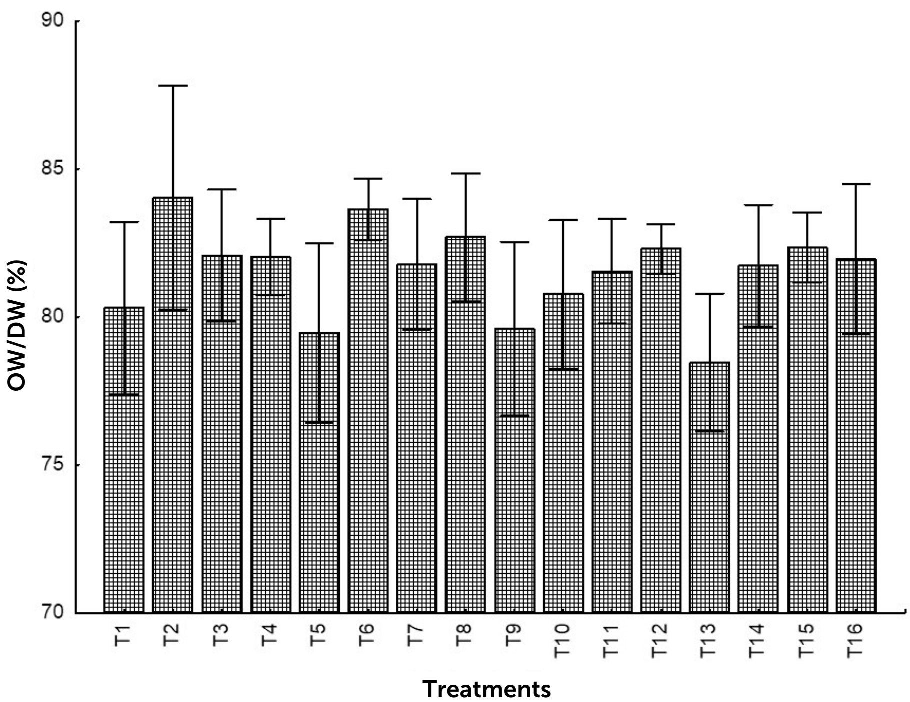

Figure 4. Percentage of organic matter with respect to dry weight (OW/DW) *L. vannamei* shrimp larvae fed with *C. muelleri* microalgae. See Table 1 for the description of the treatments. Mean ± standard deviation (n=8). There were no significant differences between treatments ($p<0.05$, two-way ANOVA).

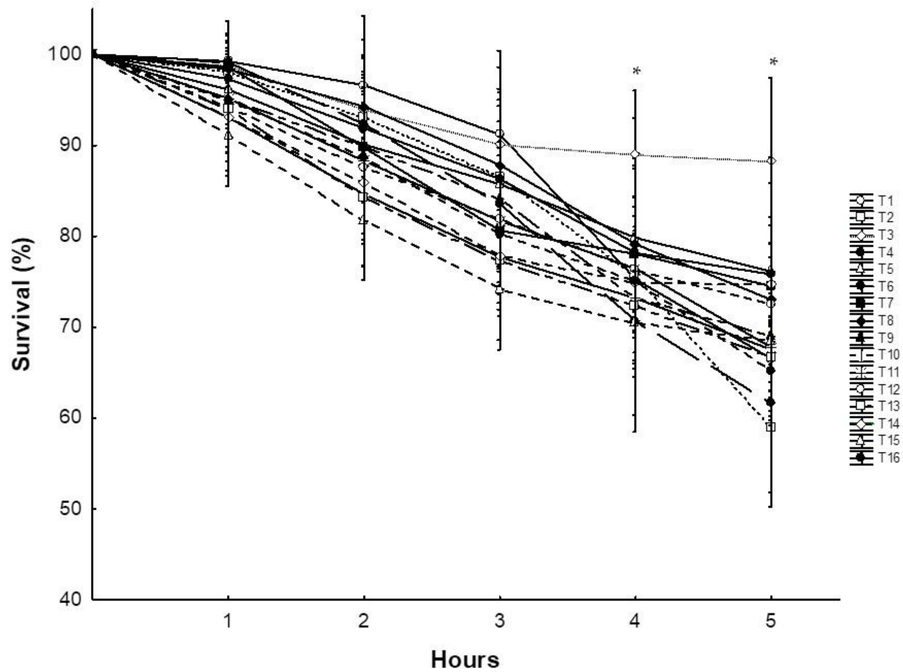

Figure 5. Survival (%) of *P. vannamei* larvae fed with *C. muelleri* microalgae.

variables. These results suggest that the larvae in these treatments were probably overfed, which could have caused an increase in toxic metabolites resulting from the metabolism of the microalgae, coupled with the decomposition of the dead matter on the bottom (Artiles-Rodríguez, 2000; Godínez *et al.*, 2005), directly affecting the optimal performance of the larvae. However, survival did not reflect a negative effect of this possible overfeeding.

Unlike previous studies with shrimp postlarvae (Otoshi *et al.*, 2006; Krummenauer *et al.*, 2011; Lorenzo *et al.*, 2016) have revealed that high densities of organisms in cultures ($\geq$300 postlarvae L^{-1}) affected their performance; in the present study, no clear effect of the density of *P. vannamei* zoea on the estimated variables was observed. Even, contrary to what was expected, T4, which was the result of the combination of the highest density of larvae (D4=400 larvae L^{-1}) and the lowest concentration of food (C1), registered similar values to the treatments with the lowest larval density. This suggests two situations: the first is that, at least during the zoea stage, it is possible to maintain cultures at high densities (400 larvae L^{-1}), and the second is that the food provided was sufficient to meet the nutritional needs of the larvae under the culture conditions used.

In experimental cultures of juveniles of *P. vannamei* juvenile (Lin and Chen, 2001) and *L. schimiti* (Barbieri, 2010), there is evidence that high mortalities are associated with high shrimp densities ($\geq$100 juveniles m^{-2}) mainly due to the high concentrations of ammonium that can be recorded in the crops. In addition to the possibility of the presence of cannibalism, associated with stress factors due to density and lack of food (Arnold *et al.*, 2005). However, in the juveniles of *P. vannamaei* cultivated in Biofloc systems, the results have been encouraging, since there has been no negative effect on the culture yield as the density of the organisms increases at densities of up to 364 juveniles m^{-2} (Neal *et al.*, 2010), as long as nutritional requirements are met and intense light or natural light is provided (Costa *et al.*, 2016). It is worth mentioning that, although the evidence of the aforementioned works does not reveal, in a statistical way, a negative effect on the survival and growth of shrimp as a result of the high densities of the cultivation, quantitatively, the highest yields of the culture were obtained at the intermediate or low densities.

CONCLUSION

The best results in relation to growth, development and survival were obtained in T3 (D300C1), whose larval density was intermediate (300 larvae L^{-1}) and the concentration of food was low. Therefore, the use of these conditions is recommended for subsequent cultures of *P. vannamei* zoea, as a way of preventing the effects associated with high densities (cannibalism, food shortages, excess of nitrogenous products) and high food concentrations (increase in production costs, excess of toxic metabolites resulting from the metabolism of microalgae, decomposition of organic matter).

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Flowering in *Hylocereus* spp.: comparative analysis and self-incompatibility

Del Ángel-Pérez, Ana Lid[1]; Nataren-Velázquez, Jeremías[1]; Megchún-García, Juan Valente[2]; Villagómez-del-Ángel, Tanith E[3]; Ayala-Garay, Alma V.[4]

[1] Campo Experimental Cotaxtla/INIFAP. Km. 34.5 Carretera-Veracruz-Cordoba, Municipio de Medellin de Bravo, Veracruz, México. C.P. 94270.
[2] Tecnológico Nacional de México/Instituto Tecnológico Superior de Jesús Carranza. Jesús, Carranza, Veracruz, México. C.P. 96950.
[3] Universidad Veracruzana/ Campus Mocambo.
[4] Campo Experimental del Valle de México/INIFAP.
* Correspondence author: juanvalente.m@itsjc.edu.mx, juan.vm@jesuscarranza.tecnm.mx

ABSTRACT

Objective: To know the variables of the reproductive component of materials for manual cross-pollination purposes, for which the search for sexual synchrony is emphasized.
Design/methodology/approach: Four materials were analyzed, two with white pulp and two with red pulp, about which it is unknown if they present sexual self-incompatibility, and the synchrony in flowering is also unknown. The flowering phase and the variables that can shed light on its reproductive behavior were analyzed
Results: It was found that the red materials present strong hercogamy, they cannot self-fertilize. There is also no floral synchrony, but there is closeness between the date of anthesis in a pitahaya with white pulp and a red one, which would allow promoting cross-pollination.
Study limitations/implications: *Hylocereus* spp. is consumed in a large part of the world and has acquired a very strong importance since the industrial demand is increasing; however, several of the genotypes used in commercial production in Mexico show low fruit set compared to high floral emission, which is considered self-incompatibility.
Findings/conclusions: The presence of hercogamy, stronger in the red-fleshed materials, indicates the existence of sexual self-incompatibility, explaining the fact that the materials emit a large number of flowers, but do not achieve fruit setting.

Keywords: Cactaceae, pollination, fruit trees.

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INTRODUCTION

Pitahaya (*Hylocereus* spp.) is a fruit tree with potential for the national and international market due to its demand for fresh consumption and industrial use, since it favors economic profitability (Centurión-Yah *et al.*, 2008), and can be a strategy to increase the contribution of the sector and to make more dynamic rural development processes, particularly in areas with agroecological limitations (Quirós-Madrigal, 2010).

Image by Antonio Jose Cespedes at Pixabay

The origin of pitahaya is attributed to the forested regions of the tropics and subtropics of Mexico, Central and South America; its cultivation is distributed throughout the world, especially because it can grow in soils with low organic matter content and nutrient deficiencies (de Oliveira *et al.*, 2020). It has climbing habits and requires a tutor, since its stems climb trees and rocks. It uses the crassulacean acid metabolism (CAM) pathway (Osuna-Enciso *et al.*, 2016), so greater water use efficiency is expected and it is exceptionally tolerant to long periods of drought so it can be cultivated in arid and semi-arid areas (Tel-Zur *et al.*, 2011).

Hylocereus spp. is found in the wild in Mexico and its seeds are disseminated by birds. It has a wide diversity and variability of materials, which generate fruits with different characteristics and qualities that can be directed to different demands of the agrifood chain. The main consumption of *Hylocereus* is as fresh fruit, but the flowers and tender shoots are also consumed as fresh vegetables and its potential for cultivation is expanding due to strong demand in the industrial, food technology and pharmaceutical areas. Within the industrial market, various authors (Esquivel & Araya, 2012; Woo *et al.*, 2011; Wong & Siow, 2015), pointed out the importance of pitahayas for their betalains, especially betanins as a natural food colorant, and pectin as a good quality thickener, as well as a colorant in the cosmetology industry (Le Bellec, 2004; Retana-Sanchez *et al.*, 2019). Al-Alwani *et al.*, 2017, replaced the use of silicon with pitahaya dye to fabricate solar cells and to generate a low-cost and environmentally-friendly sensitizer.

The demand for pitahaya has favored its commercial development in Asian countries that take advantage of windows of opportunity in the European market throughout the year, such as Vietnam and Thailand, which have hybrids, as well as Israel; the cultivation techniques are in pots in protected environments, with fertigation and cross-pollination. In Mexico, it is grown commercially in Puebla, Oaxaca, Yucatán, Campeche, Quintana Roo, Chiapas, Tabasco, Sinaloa and Veracruz with variable average yields ranging from 3.5 to 16.0 ton/ha (Martínez-Ruiz *et al.*, 2017; Osuna-Enciso *et al.*, 2016). The fruit that reaches Mexico's markets comes from small commercial plantations and orchards that use materials of wild origin or selected by the producers. One of the most important problems is the low proportion of fruits developed in relation to the flowers produced, mainly in red-fleshed pitahaya, which is associated with sexual self-incompatibility; various reports point out that some genotypes are self-incompatible, that is, incapable of self-pollinating and fertilizing with their own pollen, even though the pollen itself is viable (Cohen & Tel-Zur, 2012; Lichtenzveig *et al.*, 2000). Self-incompatibility is due to some species-specific mechanisms such as hercogamy and gynoheterostyly, which affect pollination (Márquez-Guzmán *et al.*, 2005; Weiss *et al.*, 1994). As a result, growers harvest fruit of different sizes, many of the fruits are not marketable and yields are low. To solve this problem, it is necessary to carry out manual cross-pollination, when the materials have a certain level of self-incompatibility. Strong self-incompatibility has been reported in red-fleshed genotypes, which should be pollinated with pollen from white materials.

Therefore, the flowering stage of *Hylocereus* spp. and some floral biology variables of four materials are analyzed: two of red pulp, Rojo Ch and Rojo P, from Chiapas and Puebla, which are cultivated commercially; the other two are of white pulp and were selected by INIFAP, Andrea of pink peel and Tanith of yellow peel, with high potential for commercial cultivation, about which it is unknown whether or not they have traits of self-incompatibility. The final objective of the analysis is to determine variables of the reproductive component of the materials for the purpose of manual cross-pollination, for which the search for sexual synchrony is emphasized.

MATERIALS AND METHODS

The study was carried out at two sites, first an orchard at the Cotaxtla Experimental Field in Veracruz (INIFAP) where the white materials were established, and second a farm in Suchiapa Chiapas where the red genotypes are grown. The Cotaxtla Experimental Field is located in the municipality of Medellín, Veracruz, Mexico (18° 56' 1.8" N, 96° 11' 35.5" W), with an altitude of 10 m. The average rainfall is 1336 mm, where the rainiest months are July and September and the average annual temperature is 26 °C [12]. Suchiapa is located at 16° 37' 30" latitude North and 93° 6' 0" longitude West and an altitude of 530 m. The climate is warm sub-humid with periodic rains, the average annual temperature is 28 °C, with an average rainfall of 956 mm (H. Ayuntamiento Municipal de Suchiapa, 2019).

This study analyzes the flowering stage and certain aspects of floral biology of four materials, two of red pulp (Rojo Ch and Rojo P, from Chiapas and Puebla, *H. purpusii* and *H. ocamponis*, respectively), grown commercially in Chiapas due to their high productive potential. The other two are white-fleshed and were selected by INIFAP (Andrea, of pink peel and Tanith of yellow peel, both *H. undatus*) because of their great potential for commercial cultivation. Therefore, since the interest is focused on the flowering stage, nine variables were considered: flower length, ovary length and diameter, style length and diameter, number of stigma lobes, locule length and diameter, and hercogamy distance.

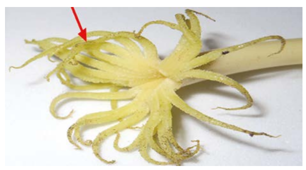
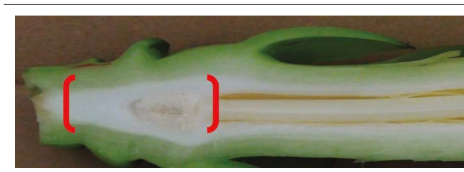
The four materials are rainfed and only manual auxiliary irrigation is used during the installation stage of the orchard in the field. Producers consider the included materials to be of great importance, to offer in the international market. Fifteen flowers of each material were used, which were sectioned to collect the data of the variables included in the analysis. Most of them come from the most abundant floral flushes, the second from the white-fleshed pitahayas, and the second and third from the red-fleshed pitahayas. Table 1 shows the variables of interest.

To help determine the sexual synchrony of the materials, four phases of the flowering stage were considered: emergence of the flower bud (when the areola begins to swell), anthesis (flower opening), fruit setting, and arrival at fruit maturity (as the end of the stage). The sequence was followed from the first flush of flower buds and during the total flushes of each material, since those with white pulp only yielded four, while those with red pulp managed to emit five.

RESULTS AND DISCUSSION

Pitahaya is a perennial plant that grows wild in southeastern Mexico and Central America. Its metabolism is crassulacean acid (CAM), which uses PEP carboxylase (an enzyme that binds CO_2 to phosphoenolpyruvate) in the fixation of atmospheric CO_2.

Table 1. Flower characteristics of the materials analyzed.

Internal characters of the flower	**Data**		
Style (cm)	**Selection**	**Longitude**	**Width**
	Tanith	25.2	0.48
	Andrea	25	0.44
	Roja Ch	23.04	0.52
	Roja P	22.95	0.50
Stigma lobe (cm)		**Longitude**	**Width**
	Tanith	2.3	26.8
	Andrea	1.8	25.8
	Roja Ch	2.3	19.6
	Roja P	2.3	25.0
Herkogamy (cm)		**Distance**	
	Tanith	1.1	
	Andrea	1.3	
	Roja Ch	2.1	
	Roja P	2.9	
Ovary region (cm)		**Longitude**	**Width**
	Tanith	6.2	3.7
	Andrea	5.3	3.8
	Roja Ch	5.6	
	Roja P	6.50	3.2
Loculus		**Longitude**	**Width**
	Tanith	1.6	1.0
	Andrea	1.5	0.9
	Roja Ch	2.1	2.6
	Roja P	1.6	1.4
Flower (cm)		**Longitude**	**Color**
	Tanith	39	White
	Andrea	38	White
	Roja Ch	34	White
	Roja P	33	White

This type of plant keeps stomata closed during the hot and dry hours of the day and opens them at night, with less loss of water vapor (Méndez *et al.*, 2009). Its nocturnal flowering and anthesis mechanism occurs only one night; the first flower flush happens from the end of April to September, but some materials offer early flower flushes from the end of March. The flowers are hermaphrodite, some white to cream colored, and in some red-fleshed materials some reddish coloration is observed in the external sepals. They open around 18:00 h and close between 10:00 and 11:00 AM; anthesis in the flower occurs from 15 to 17 hours (Valiente-Banuet *et al.*, 2007).

Therefore, pitahaya for its natural pollination requires nocturnal pollinators such as bats, which are the most efficient, and nocturnal moths and beetles are also very important although they are less efficient (Valiente-Banuet *et al.*, 2007). On the other hand, various authors point out the existence of self-incompatibility in several genotypes of *Hylocereus*, which constitutes a genetic barrier in the progamic phase that happens between the pollen and the stigma or during the development of the pollen tubes in the style, which occurs mainly in clones of *H. costaricensis* and *H. polyrhizus* (Weiss *et al.*, 1994). The same authors point out the inability of these genotypes to form fruits after self-pollination, while in *H. undatus* they found 50% of self-incompatibility.

Sexual incompatibility is the inability of a fertile hermaphrodite plant to produce zygotes after self-pollination (Nobel & De la Barrera, 2002). It is a genetic-biochemical mechanism, where the pistil rejects its own pollen, but accepts pollen from genetically different plants; it occurs to avoid inbreeding and favors cross-pollination. It should be added that, on the other hand, the distance between the stigma and the anthers, that is, hercogamy, can also decrease the probability of autogamy (Valiente-Banuet *et al.*, 2007).

Table 2 shows the description of the floral structure in the four materials considered important in the pollination phase.

Table 2 shows that there is a very strong statistical difference in eight of the variables included that differentiate the floral structure of the materials. The data of flower length indicate that due to the average size of the flower in white-fleshed materials, they are larger and have longer style length, more stigma lobes, smaller locule diameter, and lower hercogamy.

Figure 1A presents the explanatory variables on the factorial axes; it is observed that ovary diameter, flower length and style length are of greater importance in defining the white-fleshed materials (Andrea and Tanith), while number of stigma lobes and ovary length, although important, show a secondary weight that differentiates them from the

Table 2. Flower characteristics of *Hylocereus* spp. for four materials.

Changeful	Andrea	Tanith	Roja P	Roja Ch	p (0.05)
Flower length	38.0 a	39.1 a	32.8 b	34.4 ab	<0.0001
Ovary length	5.3 a	6.2 ab	6.5 b	4.4 a	<0.0001
Ovary diameter	3.8 ab	3.7 b	3.2 a	3.5 ab	0.0097
Style length	25 b	25 b	23 a	23.0 ab	<0.0001
Style diameter	0.4 a	0.5 a	0.5 a	0.5 a	0.4405
Number of lobes	25.8 b	26.8 b	25 ab	19.6 a	0.0007
Loculu length	1.5 a	1.6 ab	1.7 ab	2.1 b	0.0125
Locule diameter	0.9 a	1.1 ab	1.4 b	1.2 ab	0.0062
Herkogamy	1.3 a	1.1 a	2.9 b	2.1 ab	<0.0001

Kruskal-Wallis, 0.05. Means with different letters are statistically different.

red-fleshed pitahayas. Figure 1A also shows how the variables hercogamy, locule diameter, locule length and style diameter define a difference between Rojo P and Rojo Ch, with Andrea and Tanith. Figure 1B shows that considering the explanatory variables used, there is a very marked difference between the materials, and manages to include 98.4% of the variability. This allowed representing in different quadrants the materials, and pointing out that the white-fleshed materials are located in an opposite dimension from the red-fleshed, where Tanith and Andrea are located in the same plane and are close to each other; with certain similarities, since an area of conflict is observed where they are intertwined, that is to say, some of their explanatory variables are similar. The red-fleshed materials, in diametrically opposite planes, are not observed as close and in a way it is because they are different materials, *H. purpusii* and *H. ocamponis*.

The variables that in some way determine the strongest differences in the floral structure of the white materials are ovary diameter, flower length and style length. The Rojo P material shows as distinctive feature a very strong hercogamy (Table 1A).

Flowering phases

Figure 2 shows the phenological development of the materials during the four phases (emergence, anthesis, fruit setting and fruit maturity) and in the floral flushes of each material. The Tanith and Andrea materials only reached four flushes, the second being the one with the highest flower emission; in the case of the red pulp materials, they reached five flushes, but only the second and third emitted the highest number of flowers; however, fewer flowers took. It was also observed that new flushes appear before maturity of the previous flush.

Figure 2 shows that the two red-fleshed cultivars emitted their first flower emissions earlier than the white-fleshed pitahayas, at the end of March and beginning of April. In white-fleshed pitahayas, the Tanith cultivar emitted its first flower flush (emergence) in early May, and Andrea at the end of May. Four productive floral flushes were observed in

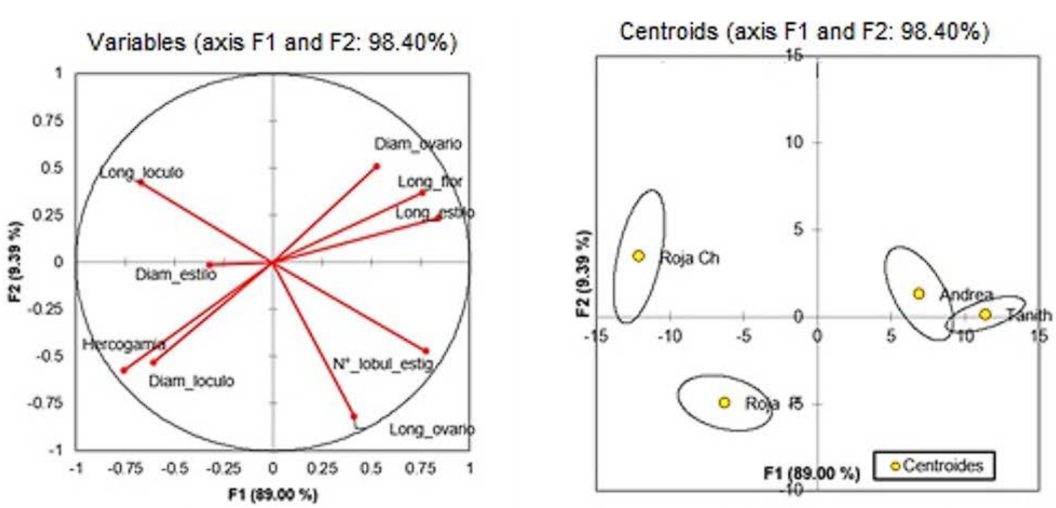


Figure 1. A: Explanatory variables and importance in the materials (p<0.0001, 0.05). B: Position of red and white materials analyzed.

Tanith and Andrea cultivars, and five in red-fleshed pitahayas. A new flush was found to emerge before fruit maturity from the previous flushes in all four cultivars.

Because the synchrony of anthesis is important for those who wish to carry out manual cross-pollination, and because the literature reports that this type of pollination improves production, since it generates larger fruits and the size of the fruit to be sent to market can be controlled. On the other hand, the literature also reports that red-fleshed materials have sexual self-incompatibility and although they offer more flower flushes than white-fleshed cultivars, self-incompatibility decreases production; on the other hand, white-fleshed materials may have partial but not total self-incompatibility. For all these reasons, it is important to know the synchrony of the materials for pollination.

Anthesis is the floral opening that occurs on a single night and is the process of growth and separation of the sepals and petals of the flowers, exposing stigmas and stamens.

The red-fleshed materials had 20-21 days from emergence to anthesis, while the white-fleshed materials had 16-17 days, an interval very close to that reported by Le Bellec (2004), of 20-21 days, and less than that found by Centurión-Yah *et al.* (2008) in Mexico of 25 to 31 days.

Figure 2 shows that no synchrony was found in the anthesis of the four materials, but there is proximity between them as in the case of Tanith and Rojo Ch during the second floral flush. Anthesis of Tanith happened on May 19 and Rojo Ch until May 21, but it is possible to refrigerate Tanith pollen at 10 °C. Another point of anthesis proximity was also between the same materials during anthesis of the third flush of Tanith on 22 July and the fourth of Rojo Ch on 21 July. Finally, the fourth flush of Tanith, on August 21, showed close proximity with the fifth flush of Rojo Ch on August 23. Flower drop was observed after self-pollination (free pollination) in red-fleshed pitahaya plants, which affected yield; on the other hand, the quality (appearance and size) is low because the fruits are of different sizes with a tendency to very low weight.

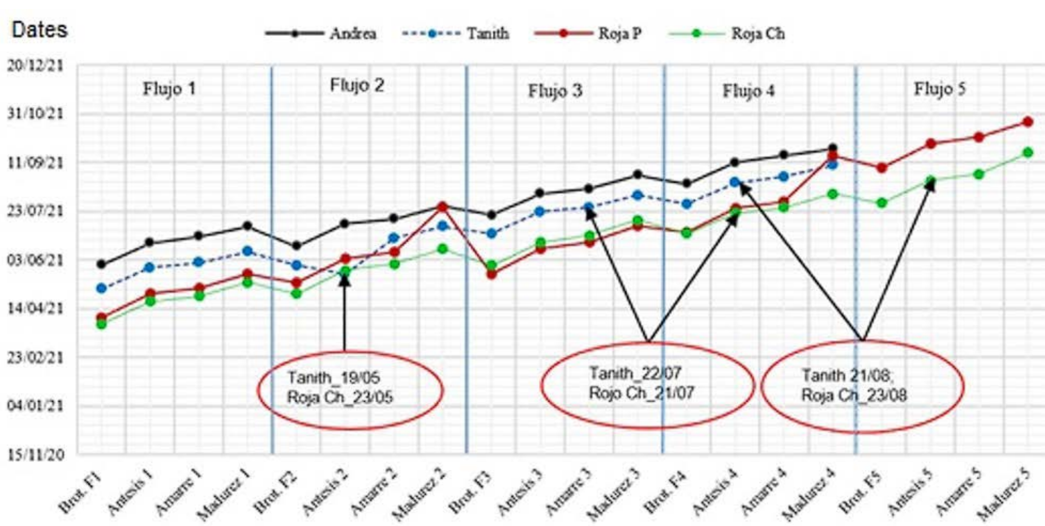


Figure 2. Phases of the flowering stage in the materials analyzed.

The other materials did not show proximity in the occurrence of anthesis, which is very important for those who wish to increase fruit setting by cross-pollination of materials. The data suggest the importance of working with more materials to ensure cross-pollination between those of white and red flesh. All pitahayas, both white and red-fleshed, reached anthesis and fruit setting at the flush rates presented, with adequate average night, minimum and maximum temperatures. The days from emergence to anthesis during the first floral flush in the white-fleshed materials was 22 days, while in Rojo Ch it was 23 days and in Rojo P 20 days, and for the white-fleshed pitahayas (*Hylocereus undatus*) it was 22 days (Figure 3). This interval was greater than that reported by Osuna *et al*. (2016) who found that on the Pacific coast, the flowers of *H. undatus* reached anthesis in 2 weeks and in autumn in three weeks. In this case, the first floral flush of the red-fleshed genotypes (*Hylocereus* spp.) was early, and emergence began at the end of March and beginning of April with minimum night temperatures of 12 °C for Rojo P and 21 °C for Rojo Ch, and 23.5 °C for white-fleshed pitahayas. On the other hand, the same authors point out that flowering is associated with an increase in temperature and relative humidity, and according to (Ortiz, 1995), for *Hylocereus* to flower it requires 10 to 12 hours of light per day and temperatures of 21 to 35 °C. Figure 3 shows the average, minimum and maximum night temperatures during floral emission during each of the flushes. Only the Rojo P genotype developed at very low temperatures, but which exceeded the survival limit of the plants.

The temperature favored the materials in some way, since above 44 °C, pitahaya plants suffer physiological stress, while the base temperature for plant survival is 7 °C. It can be said that during the productive phase they had a favorable condition to assimilate CO_2, since according to Nobel and de la Barrera, (2002), for the case of *H. undatus* a maximum net total CO_2 uptake is achieved at night temperatures averaging 20 °C, since the highest absorption happens at that time.

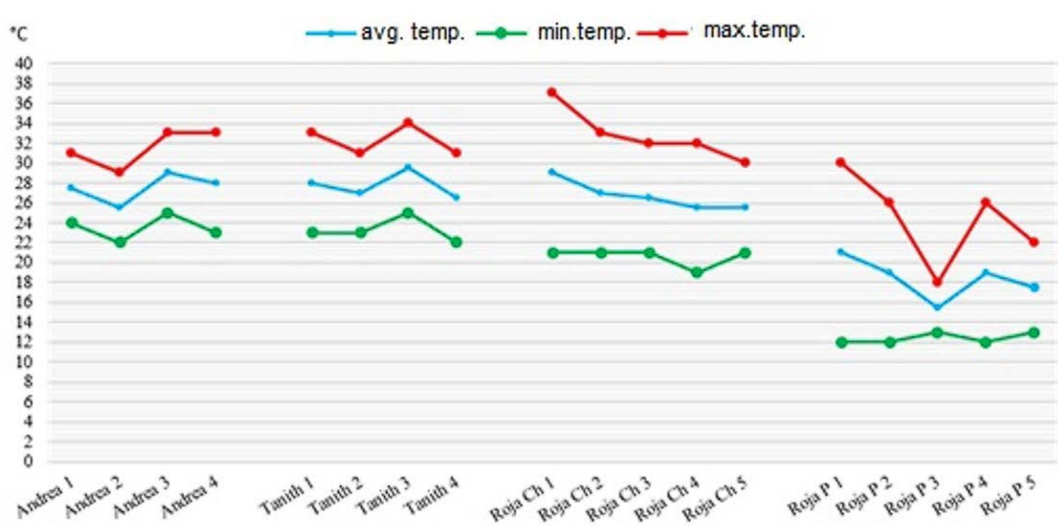


Figure 3. Nighttime temperatures of the pitahaya materials analyzed.

Production achieved by open pollination

The pitahaya production system in Mexico has evolved from backyard to traditional and semi-technified commercial production, although rainfed planting with auxiliary irrigation is predominant. For producers, obtaining the fruit is the most important objective, since their survival depends not only on the harvest but also on the continuity of the crop, whatever it may be. Among the problems encountered in the cultivation of pitahaya, particularly the red-fleshed genotypes, is the fall of flowers and small fruit. Red genotypes emit abundant flowers, but flower setting differs in each material (Figure 4).

Figure 4 shows the average number of flowers emitted per plant in the cycle, as well as the percentage of setting; less setting is observed in the red-fleshed materials, despite the fact that they emit more flowers than the white-fleshed ones; the latter achieved 94% setting, while the red ones achieved less than 45%. There is a need to increase the percentage of setting of red-fleshed genotypes due to their high industrial demand. In general, the average number of fruits in *Hylocereus* spp. was 45 fruits per plant.

The average weight showed significant statistical difference, considering for marketing purposes, but the cultivars Tanith and Andrea stand out, since the cultivars Rojo Ch and Rojo P achieved a lower average weight, although it is possible to improve the fruit size with manual pollination. The ° Brix variable is lower in white-fleshed pitahayas, with the Tanith cultivar standing out with the lowest level and Rojo P with the highest °Brix.

Table 3. Characteristics of fruits of the materials analyzed.

Genotype	Average weight (g)	° Brix
Roja P	322.4 a	14.4 b
Andrea	366.6 ab	12.4 ab
Roja Ch	360.8 ab	13.1 ab
Tanith	413 b	11.2 a
P (0.05)	0.0244	0.0003

Kruskal-Wallis, 0.05. Means with different letters are statistically different.

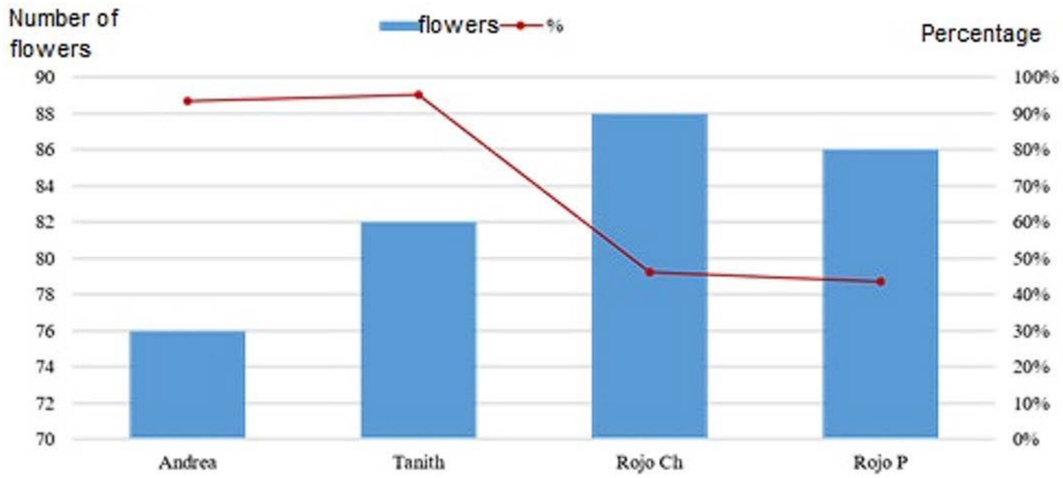


Figure 4. Flower emission and percentage of setting in each of the materials analyzed.

CONCLUSIONS

The analysis of the flowering phase of the materials shows that the variables used allow differentiating the materials, so the absence of synchrony indicates that these materials should not be planted together in an orchard when cross-pollination is sought. Only some proximity was observed in the anthesis of Tanith and Rojo Ch, so if there is the intention of planting these materials it is convenient to collect the pollen from Tanith and store it at 4 °C to pollinate Rojo Ch.

The presence of hercogamy, stronger in the red-fleshed materials, indicates the existence of sexual self-incompatibility, explaining the fact that the materials emit a large number of flowers, but do not achieve fruit setting. White pulp materials have weak self-incompatibility.

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Characterization and *in vitro* spread of mesquite (*Prosopis laevigata* (Humb. & Bonpl. ex Willd.) M.C. Johnst.)

Ortiz-Robles, María D.[1]; **Gómez-González, Adrián**[2]; **Flores-Benítez, Silvia**[1]; **Valera-Montero, Luis L.**[1]; **Romero-Amador, Lourdes D.**[1]; **Perales-Segovia, Catarino**[1]; **Silos-Espino, Héctor**[1]

[1] TeCNM-Campus El Llano, Aguascalientes, Carretera Aguascalientes-San Luis Potosí km 18, El Llano, Aguascalientes, México, C. P. 20330.
[2] Colegio de Postgraduados, Campus San Luis Potosí, Iturbide 73, Salinas de Hidalgo, San Luis Potosí, México, C. P. 78600.
* Correspondence: silosespino@hotmail.com

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ABSTRACT

Objective: To analyze fruit characteristics, genetic diversity, and *in vitro* spread of mesquite genotypes (*Prosopis laevigata* (Humb. & Bonpl.) ex Willd.) from the plains of Ojuelos-Aguascalientes, Mexico.

Design/Methodology/Approach: Fruit and leaf samples were collected from 20 mesquite genotypes to analyze and extract DNA to determine their diversity using RAPDs. Simultaneously, *in vitro* spread tests were performed.

Results: The Ojuelos de Jalisco genotype stood out for the weight of its fruits, while La Presa genotype stood out for its degrees Brix. Genotypes were grouped according to their best growth condition for their genetic analysis. In *in vitro* spread, AG_3 with IBA allowed stem/shoot elongation and root formation; meanwhile, $AgNO_3$ prevents leaf fall, allowing rooting and transfer to the soil.

Study Implications/Limitations: This study about mesquite (*P. laevigata*) was limited to the plains of Ojuelos-Aguascalientes.

Findings/Conclusions: Mesquite plants from the Ojuelos-Aguascalientes subregion, Mexico, were identified and georeferenced; likewise, a methodology for its *in vitro* spread was developed.

Keywords: Microspread, pods, *Prosopis laevigata*, DNA, georeferencing.

INTRODUCTION

Mesquite (*Prosopis laevigata* (Humb. & Bonpl. ex Willd.) M.C. Johnst.) belongs to the Fabaceae family and is a forest resource found in the arid and semi-arid zones of Mexico. It is threatened by irrational exploitation resulting in soil and drainage basin deterioration, as well as in the low food availability for wildlife (Rodríguez-Sauceda *et al.*, 2014). Eleven

Image by Michael at Pixabay

species of mesquites can be found in Mexico and *P. laevigata* is distributed in the north-central region of the country (Palacios, 2006). These species have different morphological and genetic characteristics (Luna-Castañón, 2014). Determining their genetic diversity and developing technology for their efficient management may allow a sustainable management of mesquite populations (Ríos-Saucedo *et al.*, 2011). Seeds are commonly used for its reproduction. *P. glandulosa* Torr. seeds have a rhomboid-flattened shape, are yellow-brown, measure 4.21×6.97 mm, have a 0.34 g average weight, a 62% viability, and 51% *in vivo* germination (Villareal-Garza *et al.*, 2013). Using the plant tissue culture technique, explants (stems) of *P. laevigata*, *P. glandulosa*, and *P. limensis* adult plants become necrotic and/or have a poor response; therefore, *in vitro* germination is preferred (Buendía-González *et al.*, 2007; Trejo-Espino *et al.*, 2011). Recently, *P. pallida* seeds subjected to a water immersion treatment at 80 ° C for 10 min recorded a 36.7% germination within 30 days, 2.4 shoots per axillary bud, 3.6 axillary nodes for each cultivated apical bud, 53.3% rooting of micro cuttings, and 100% acclimatized seedlings (Rivera-Curi *et al.*, 2020). Clearly, each species has specific morphological, genetic, and spread response behavior. Therefore, this work analyzed the pod characteristics and the DNA profile using RAPDs and tests were performed to establish the *in vitro* spread of mesquite from the Ojuelos-Aguascalientes subregion.

MATERIALS AND METHODS

Mesquite plants

The sampling was carried out in the subprovince of the Llanos de Ojuelos-Aguascalientes (Mexico's Mesa Central) identified as: typical plateau (43.77%), desert plain with rocky or cemented soil (22.86%), hillocks at the foot of mountains (16.30%), desert plain (14.20%), old alluvial hillocks (2.07%), and sierra with plateaus (0.80%) (INEGI, 2009). Mesquite plants adjacent to access roads were selected and georeferenced (Table 1).

Morphological analysis of pods

Fifty pods —which can be used for food— were collected per tree; then, length, diameter and weight were determined.

Genetic analysis of mesquite plants

The CTAB method (Doyle and Doyle, 1987) was used to extract DNA from fresh leaves. DNA quality was verified with agarose gel electrophoresis (1.5%), using 1X TBE buffer at 80 volts for 1.5 h, stained with ethidium bromide, and observed using an imaging system (BioDoc-ltTM Imaging System).The RAPD technique (Williams *et al.*, 1990) was used for the analyses, with 17, 10, 4, and 4 primers from the Operon A, B, F, and G series, respectively. The 12.5-μL reaction mixture consisted of 5 μL of sterile double-distilled water, 6.5 μL of reaction mixture (Gotaq® Green Master Mix), 0.5 μL of each primer (10 pmol), and 0.5 μL of genomic DNA (10 ng μL^{-1}). The terms of the reaction were the following: one cycle at 95 °C for 5 min; 40 cycles at 95 °C for 30 s, 36 °C for 30 s, 72 °C for 1 min; and one cycle at 72 °C for 4 min. For the amplification reactions, the Techne® Endurance TC-512 Gradient Thermal Cycler equipment was used. The

Table 1. Georeferenced location of mesquite plants in the Ojuelos-Aguascalientes subregion, Mexico.

Number	Community	Latitude (N)	Longitude (O)	Altitude (m)
1	Ojuelos de Jalisco	21° 52' 37.65"	101° 36' 19.45"	2237
2	Matancillas	21° 53' 16.02"	101° 38' 56.69"	2193
3	Chinampas	21° 47' 58.16"	101° 40' 36.79"	2098
4	La Presa	21° 46' 36.11"	101° 49' 49.38"	2094
5	La Troje	21° 45' 27.99"	101° 54' 27.22"	2069
6	Los Vergeles	21° 46' 08.56"	101° 56' 31.36"	2037
7	El Tepetatillo	21° 47' 21.18"	102° 00' 03.08"	2001
8	La Loma	21° 48' 47.25"	102° 04' 32.27"	1997
9	ITEL	21° 49' 13.75"	102° 06' 06.95"	2009
10	El Retoño	21° 50' 18.62"	102° 09' 15.15"	2013
11	Norias de Paso Hondo	21° 51' 18.64"	102° 12' 15.24"	1980
12	La Pona	21° 53' 18.93"	102° 16' 03.32"	1896
13	Pirules	21° 51' 45.65"	102° 19' 31.86"	1844
14	Isla San Marcos	21° 51' 46.66"	102° 19' 15.93"	1840
15	Ciénega Grande	22° 11' 24"	102° 01' 20"	2150
16	Los Campos	22° 01' 41"	101° 50' 34"	2110
17	Cerro de Montoro	22° 01' 29"	101° 49' 54"	2112
18	El Rusio (Los campos)	22° 01' 39"	101° 50' 44"	2110
19	Pabellón de Arteaga	22° 06' 17"	102° 18' 16"	1900
20	San Francisco de los Romo	22° 01' 18"	102° 13' 20"	1880

generated fragments (RAPD) were used to determine the profiles of the mesquite plants, a similarity matrix was calculated with the Bray-Curtis coefficient, and cluster analyzes were performed with the arithmetic mean method, using NTSYS pc 2.1 (Exeter Software, Setauket, New York, U S A) and Past 2.03.

In vitro spread procedure

Stem segments (1-2 cm) of 4-year old plants (established under nursery conditions) and seeds (mechanically scarified, 12-hour fungicide immersion), collected from plants analyzed in the morphological and genetic study were used, based on the following protocol: immersion in 3 g·Captan® L^{-1} for 30 min, 1.2 g·Benomyl® L^{-1} for 30 min, 0.05% colloidal silver for 10 min, 0.8% Cloralex® for 15 min, hydrogen peroxide (1:1, v:v) for 15 min, and finally 70% alcohol spray (at every change, the plants were rinsed with sterile distilled water). Stem explants were subjected to disinfectants, fungicides, and bactericides at different times and concentrations. The Murashige and Skoog (1962) basal medium was used with cefatoxime (350 mg·L^{-1}), growth regulators (BAP, 2,4-D, kinetin, and ANA) and antioxidants (silver nitrate). Then, 6.5 g of agar were added to the culture medium and it was sterilized at 121 °C for 15 min. Once the explants were established, they were transferred to the incubation room with temperatures from 20 to 28 ° C and a 16-h photoperiod of light. Rooted explants were established in previously sterilized substrate and placed under greenhouse conditions.

RESULTS AND DISCUSSION

Mesquite plants and their fruits

Twenty plants with clear morphological differences were chosen from nearby trees (height, diameter, leave and pod size, and growth pattern). In relation to the fruit (pods), the genotypes that stood out for their weight came from Ojuelos, Jalisco (570 g in 50 pods), while the tree of Los Vergeles from Lagos de Moreno, Jalisco stood out for its length and number of seeds per pod (18.88±2.50 cm and 24±1.62 seeds). The pods from Matancillas (Ojuelos, Jalisco) had a larger diameter (1.8±0.13 cm). The highest sugar concentration (° Brix) was found in the sample collected in La Presa (Lagos de Moreno Jalisco) (Table 2). Determining the mesquite pods characteristics allows us to infer their potential use as forages (Armijo-Nájera *et al.*, 2019), timber, or as food for human consumption. For instance, García-López *et al.* (2019) reported a mean production of mesquite pod (*Prosopis laevigata*) of 3.7 ton·ha^{-1}, which was considered good, given the unfavorable precipitation and temperature conditions prevailing in the Altiplano Potosino.

Mesquite characterization by PCR-RAPDs

Out of the primers tested, only OPA16 (5'AGCCAGCGAA3) and OPG04 (5'AGCGTGTCTG3') were amplified (Figure 1A). Overall, fragments were amplified 250-

Table 2. Morphological characteristics of mesquite pods collected in Mexico's Mesa Central, Los Llanos de Ojuelos-Aguascalientes subprovince, Mexico.

Collection site	Weight of 50 pods (g)	Length (cm)	Diameter (cm)	Seeds per pod	°Brix
Ojuelos de Jalisco	570.0	17.6±1.80	1.7±0.15	20±1.33	15.5±1.3
Matancillas	433.8	14.2±1.86	1.8±0.13	18±1.93	27.0±1.3
Chinampas	294.2	16.2±1.85	1.3±0.23	16±2.94	18.0±1.1
La Presa	383.4	13.9±2.76	1.0±0.36	18±2.60	38.0±1.2
La Troje	120.2	9.4±1.75	1.3±0.45	9±2.77	20.0±0.8
Los Vergeles	272.2	18.8±2.50	1.6±0.19	24±1.62	25.0±1.8
La Loma	318.6	15.6±1.67	1.5±0.31	17±2.27	28.4±0.8
El Retoño	140.0	9.9±1.35	1.3±0.33	15±1.63	22.0±1.3
ITEL	290.6	15.7±1.66	1.0±0.30	15±1.94	17.5±0.7
La Pona	280.4	13.0±2.08	1.6±0.32	20±2.58	12.0±0.6
Norias de Paso Hondo	fruitless				
Isla San Marcos	269.0	18.2±1.95	1.5±0.32	20±1.58	11.5±0.1
Pirules	190.6	10.6±1.46	1.0±0.29	16±3.06	20.5±0.5
El Tepetatillo	294.4	18.0±1.33	1.4±0.47	19±1.60	21.5±0.6
Ciénega Grande	141.6	9.7±1.46	1.0±0.36	14±1.56	21.0±0.2
El Rusio (Los Campos)	290.0	15.3±2.04	1.3±0.35	21±1.79	25.0±0.9
Los Campos	235.0	12.0±1.18	1.2±0.40	14±1.70	25.5±0.5
Cerro de Montoro	250.4	14.8±0.94	1.0±0.36	20±1.89	20.5±0.9
Pabellón de Arteaga	290.6	15.03±2.05	1.4±0.36	18±3.40	19.0±0.4
San. Fco. de los Romo	395.0	17.9±1.25	1.5±0.25	22±1.33	7.0±0.3

2000 bp samples; La Loma and Norias de Paso Hondo specimens and Los Vergeles and El Retoño specimens showed an additional >2000 bp fragment with the OPA16 primer and the OPG04 primer, respectively. All the specimens coincided with two fragments: the first of 1000 and the second of approximately 1900 bp. A dendrogram was generated with the amplified fragments (Figure 1B) consisting of two large groups, distributed per agroecological region: one (A) that included three genotypes (Ojuelos de Jalisco, El Retoño, and El ITEL) and another composed of two subgroups (B) made up by seven genotypes (BI, Ciénega Grande, Chinampas, La Loma, Norias de Paso Hondo, Los Campos, and Cerro de Montoro) and eigh genotypes respectively (BII, Matancillas, El Rusio (Los Campos), Pirules, Los Vergeles, El Tepetatillo, San Marcos, La Pona, and La Presa). The Norias de Paso Hondo (fruitless) genotype showed an additional fragment that was not found in all

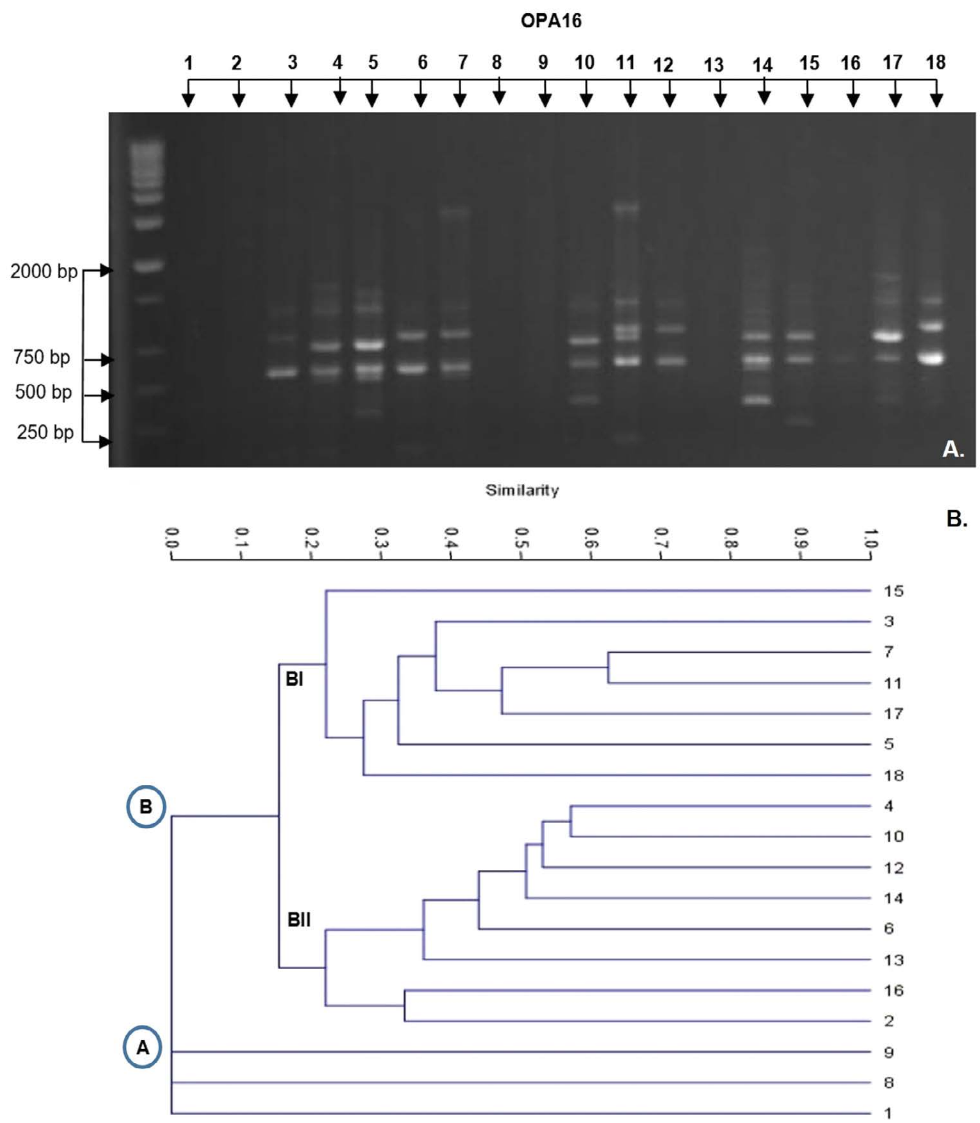

Figure 1. Amplification of DNA fragments by RAPDs in mesquite (*Prosopis laevigata*) from the plains of Ojuelos-Aguascalientes. A) Fragments with OPA16 and OPG04 primers. B) Corresponding dendrogram with groups A and B in blue circles.

other genotypes. Similarly, Luna-Castañón (2014) found a morphological difference in the same population (progenies vary in seedling height and the length-width of petiole, pinna, and pairs of leaflets), as well as a genetic difference between plants that are representative of the state of Aguascalientes. Morphometric and genetic diversity studies were carried out to identify salt tolerant *Prosopis* species (Roser *et al.*, 2014). The aim ot those studies was to design conservation and use strategies (Anand *et al.*, 2017) that help to identify threatened species (Moncada *et al.*, 2019; Contreras *et al.*, 2020) and to determine the species origin (Contreras-Negrete *et al.*, 2021). This study seeks to find mesquite genotypes with an outstanding characteristic, focusing on the fruit of wild plants.

Mesquite *in vitro* spread

The following asepsis protocol was used: Roma® detergent (5 g L^{-1}), 30% alcohol, Cloralex® (0.4%) and Cupravit® (3 g L^{-1}) for 5 min. As a result, an 85% asepsis was obtained from stem explants, which generated 5.6 (±1.5) shoots, as well as a 100% callus formation (Figure 2A) with MS (1962), with 2,4-D (3 g L^{-1}), ANA (2 g L^{-1}), and BAP (5 g L^{-1}). Unfortunately, explants became necrotic. With the *in vitro* seeds, 100% germination was achieved within 8 days (Figure 2B, 45-day plant).

The *in vitro* germinated explants also responded differently (Table 3), depending on the culture medium composition. In general, AIB generated oxidized callus, shoots, and roots. 2,4-D formed small necrotic calluses around the base. ANA induced an 80% callus formation, without shoots and roots. AG_3 with IBA promoted stem and shoot elongation, as well as root formation (Figure 2C). Regarding BAP, an 80% necrotic callus was formed and there was a low shoot formation and a lack of root formation (Figura 2D). Kinetin promoted callus (70%) and shoot formation (80%) but did not form roots. $AgNO_3$ prevented leaf fall (Figure 2E). Finally, the formed plants were established in soil and then transferred to greenhouse conditions (2F). The values obtained in this study are lower than those reported by Buendía González *et al.* (2007) for *P. laevigata* (MS with 2,4-D+BA); meanwhile Maldonado-Magaña *et al.* (2013) cultured cells in suspension with MS medium (1962) at half concentration with 2,4-D and kinetin. Minchala-Patiño *et al.* (2014) used the

Table 3. Behavior of mesquite explants in 100% MS to which growth regulators and antioxidant agents were added.

mg L^{-1}	Callus		Shoots		Root	
	(%)	Length (cm)	(%)	Length (cm)	(%)	Length (cm)
IBA (2)	100	3.29±1.90	43	1.45±2.43	33.25	0.26±0.20
2,4-D (3)	50	0.81±0.26	0.0	0.0	0.0	0.0
ANA (0.2)	100	0.7±0.2	0.0	0.0	0.0	0.0
AG_3 ((Pri-vera)	40	0.74±0.33	27.5	1.65±1.2	0.0	0.0
BAP (X)	100	0.7±0.2	10	0.1±0.0	0.0	0.0
KIN (0.2)	100	0.6±0.2	40	0.4±0.2	0.0	0.0
KIN+AG_3 (2/4)	100	1.08±0.35	70	1.08±0.83	73.3	2.18±0.58
$AgNO_3$	100	0.58±0.3	45	0.4±0.3	86	1.5±0.9

Number of repetitions: 10.

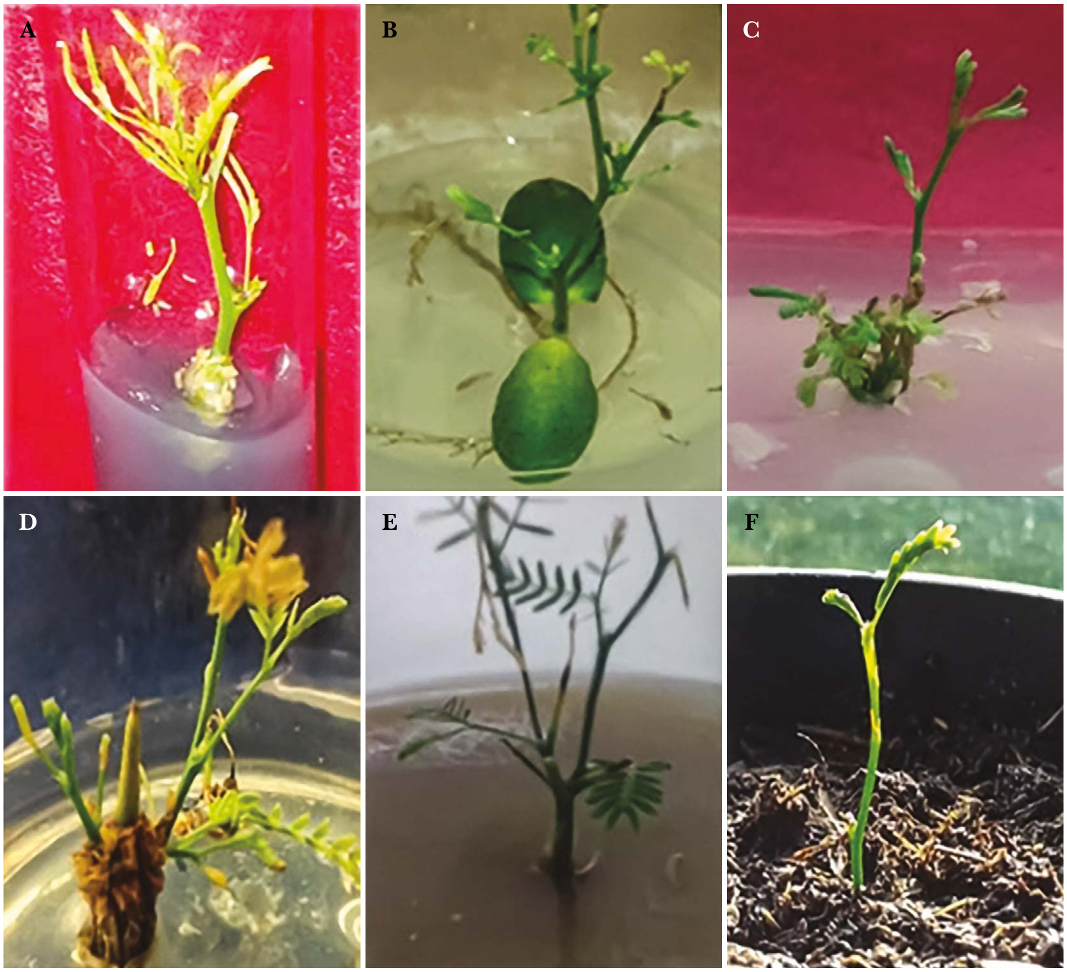

Figura 2. Evidence of the *in vitro* spread of mesquite (*Prosopis laevigata*) from the subprovince of Los Llanos de Ojuelos-Aguascalientes. A. Asepsis of stem explants generated shoots, as well as callus formation. B. *In vitro* seed germination. C. AG_3 with IBA elongated stems and shoots and formed roots. D. BAP formed necrotic callus, low shoot formation, and no root formation. E. $AgNO_3$ prevented leaf fall.

kinetin+adenine sulfate combination at a rate of 1 and 25 g·L^{-1}. Recently, Rivera-Curi *et al*. (2020) obtained good results (number of shoots and roots) with the *P. pallida* species in a cytokinin-free medium, putting cotton on the lid to remove ethylene; for its part, 0.5 mg IBA L^{-1} was enough to achieve rooting.

CONCLUSIONS

In this research, mesquite (*P. laevigata*) genotypes were characterized based on fruit size and DNA profile grouped by RAPDs, obtaining a methodology for *in vitro* spread. The Ojuelos of Jalisco, Los Vergeles, Matancillas, and La Presa genotypes were remarkable. The genetic profile grouped the individuals according to the proximity and/or orographic physical barrier of the studied subprovince. Within *in vitro* spread, AG_3 with IBA resulted in the elongation of stems, shoots, and roots, while $AgNO_3$ prevented leaf fall, achieving rooting and transfer to soil. Therefore, we infer that, even though mesquite populations belong to "the same species", the cross-pollination process enables a great interaction between individuals in their natural environment.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Sensory perspectives of the consumption of chips-type snacks made from black bean (*Phaseolus vulgaris* L) and peanut (*Arachis hypogaea* Linn) flours by university students in the Toluca Valley

Robledo-Ramírez, L.H.[1]; Bautista-Mora, G.N.[1]; Domingo-Gonzales, A.K.[1]; Flores-Rodríguez, M.L.[1]; Ramírez-Peñaloza, S.[1]; Cruz-Monterrosa, R.G.[2]; González-Martínez, A.J.[3]; Martínez-Arellano, I.[4]; Álvarez-Cisneros, Y.M.[5*]

[1] Licenciatura en Ciencia y Tecnología de Alimentos. División de Ciencias Biológicas y de la Salud. Universidad Autónoma Metropolitana Unidad Lerma. Av. De las Garzas 10. Col. El Panteón, Lerma de Villada, Estado de México. C. P. 52005.

[2] Departamento de Ciencias de la Alimentación, División de Ciencias Biológicas y de la Salud. Universidad Autónoma Metropolitana, Unidad Lerma. Av. de las Garzas No. 10, Col. El Panteón, Municipio Lerma de Villada, Estado de México, C.P. 52005.

[3] Oficina de Planeación e Información Institucional, Rectoría de Unidad. Universidad Autónoma Metropolitana, Unidad Lerma. Av. de las Garzas No. 10, Col. El Panteón, Municipio Lerma de Villada, Estado de México, C.P. 52005.

[4] Instituto de Ciencias Aplicadas y Tecnología, Universidad Nacional Autónoma de México, Circuito Exterior S/N, Ciudad Universitaria, 04510, Ciudad de México, México.

[5] Departamento de Biotecnología, Universidad Autónoma Metropolitana, Unidad Iztapalapa, Av. Ferrocarril de San Rafael Atlixco 186, Col. Leyes de Reforma, 1[a]. Sección. Alcaldía Iztapalapa. C.P. 09310, Ciudad de México, México.

* Correspondence: acym@xanum.uam.mx

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ABSTRACT

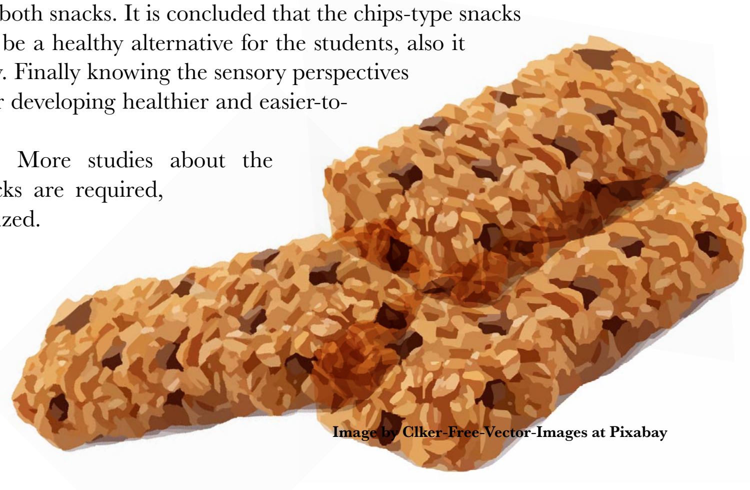
Objective: The objective of this study is to evaluate the sensory perspectives of chips-type snacks made from black bean dry (*Phaseolus vulgaris* L) and peanut (*Arachis hypogaea* Linn) flours in a university population.

Design/methodology/approach: Two formulations were realized from peanut and black bean previously toasted and milled: 1) (PF) 50% peanut flour, 12.5% rice flour and 12.5 % cornstarch; 2) (BbF) 50% black bean flour, 12.5% wheat flour and 12.5% cornstarch. Each mixture of flours was extruded and fried until obtained chips-type snacks. A statistical-descriptive study was carried out from the observation of the eating habits of university students in the State of Mexico. On other hand, an affective test was tested to evaluate the acceptance of chips-type snacks made with PF and BbF in the categories of odor, taste, hardness, and easy to break.

Findings/conclusion: According to the questionnaire, the female gender has higher snack consumption than the male population. On another hand, potato chips are in third place as the most consumed snacks for women they preferred to consume them in their houses. Peanut and chickpea flour are the most preferred by the female population. There was no significant difference ($p>0.05$) between the snacks prepared with PF and BbF, but 60% of the consumer accepted both snacks. It is concluded that the chips-type snacks made with different formulations could be a healthy alternative for the students, also it kind of products have good acceptability. Finally knowing the sensory perspectives could provide important information for developing healthier and easier-to-eat snacks.

Limitations on study/implications: More studies about the characterization of the chips-type snacks are required, also a mix of both legumes could be realized.

Keywords: bean, peanut, chips, snacks, sensory perspectives.



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INTRODUCTION

Consumer demand for ready-to-eat foods is increasing due to the need for timesaving in the modern lifestyle, also they want healthy and nutritional foods (Pasqualone *et al.*, 2020; Arise *et al.*, 2021). One alternative to this demand is "snack foods" which provide the sense that the appetite is satisfied. It is a small portion of food consumed between meals and snacking could be a habit of young and old people because it supplies the calories needed during an active day, or it is just eaten for pleasure (Alamu *et al.*, 2020; Hess *et al.*, 2016). Some examples of snacks are cookies, cakes, sugar-sweetened beverages, and chips. Recent studies also indicate that legume flour can be incorporated into snacks instead of cereals, significantly improving their functional properties and their nutritional value (Szymandera-Buszka *et al.*, 2021). Legumes are highly beneficial in the diet of people, due they have considerable quantities of water-soluble vitamins, minerals, and bioactive ingredients such as polyphenols, essential amino acids, fibers, and proteins. Also, they are lower in levels of saturated fat, glycemic index (GI) (ranging from 16 to 30), salt, and sugar (Neder-Suárez *et al.*, 2021; Pasqualone *et al.*, 2020; Szymandera-Buszka *et al.*, 2021; Tas & Shah, 2021). Also, it is a cheaper source of protein and energy, particularly for rural populations (Arise *et al.*, 2021). The term legumes originated from the Latin word "*Legere*", which refers to those plant species whose fruits are enclosed in pods and belong to the *Fabaceae* or *Leguminosae* family. The main legumes consumed by humans such as alfalfa, pea, bean, chickpea, beans, green beans, lentils, peanuts (groundnuts), and soybeans. Pulses are the edible seeds of plants in the legume family, all pulses can be called legumes, but not all legumes are pulses (Tas & Shah, 2021).

The bean (*Phaseolus* spp.) is a pulse that has spread practically throughout the world and is considered part of the basic diet of a large part of the population. In the western region of Mexico occurred the domestication of the common bean (*Phaseolus vulgaris* L.), so it is the second most-consumed legume, according to the INEGI in 2019. The annual bean production was 828, 113.9 tons, and currently, 70 species of the 150 existing worldwide have been reported in Mexico and five of them have been domesticated (*P. vulgaris* L., *P. coccineus* A. Gray, *P. lunatus* L., *P. acutifolius* A. Gray, and *P. dumosus* Mac Fady) (Lépiz, 2007). Regarding its nutritional content, the protein content varies from 14 to 33%, being rich in amino acids such as lysine (6.4 to 7.6 g/100 g of protein) and phenylalanine+tyrosine (5.3 to 8.2 g/100 g of protein), but with deficiencies in methionine and cysteine, despite this, the quality of cooked bean protein can become up to 70% compared to a protein of animal origin. Also, it has high concentrations of vitamins such as thiamine, riboflavin, and niacin and minerals such as phosphorus, iron, and zinc (Espinoza-García *et al.*, 2016; Ulloa *et al.*, 2011; Figueroa *et al.*, 2015). On the other hand, peanuts (*Arachis hypogaea* Linn), known as groundnut, are botanically defined as legumes and it is the main legume consumed worldwide, having great economic importance in countries such as China, India, and the USA (Wang *et al.*, 2021). Peanuts are rich in unsaturated fatty acids, phytosterols, and other bioactive such as fibers, tocopherols, all the essential amino acids, and phenolic antioxidants compounds. Also, it is an important source of high-quality protein (22-30%), carbohydrates (15-21%), fats (42-49%), vitamins B and E, minerals such as iron, zinc, potassium, and magnesium, antioxidant minerals (selenium, manganese, and copper), and fiber content (8.5% approximately) (Arya *et al.*, 2016; Bravo *et al.*, 2018; Guo *et al.*, 2020). According to the Protein Digestibility Corrected Amino Acid Score (PDCAAS), peanut proteins are nutritionally equivalent to meat and eggs, also it is the biggest source of arginine (FAO, 2002). According to INEGI, fried snacks generate Mexico a market of 62,758 million pesos, which corresponds to a production of 22,558 tons, and at the end of 2014, the country consumed on average 157,000 tons of chips per year. So, chips-type snacks are the most consumed by young university students. For all of these, the objective of this study is to evaluate the sensory perspectives of chips-type snacks made from bean dry (*Phaseolus vulgaris* L) and peanut (*Arachis hypogaea* Linn) flours in a university population in the Toluca Valley.

MATERIALS AND METHODS

Raw material

Peanut (*Arachis hypogaea* Linn) and Black bean (*Phaseolus vulgaris* L) were acquired in the local market "Garis", Mexico. The bean was washed and cooked with water and salt for 40 min in a pressure cooker, then dried in an oven (Hamilton beach, Virginia, EE. UU.) at 180 °C for 4 h. Finally, they were toasted at 100 °C for 15 min. The peanut was purchased previously toasted. Both were stored at room temperature until use.

Preparation of chips-type snacks

Peanut and black bean toasted were individually milled in a blender (Oster, Mexico) and sieved using a 0.4 mm mesh to obtain uniform particles. The peanut formulation (PF) contained 50% peanut flour, 12.5% rice flour, and 12.5% cornstarch. The black bean formulation (BbF) contained 50% black bean flour, 12.5% wheat flour, and 12.5% cornstarch. All mixtures were added with 1.5% salt and water was added until a malleable paste was obtained. PF and BbF flours were blended to form a thick paste, which was then extruded and fried using a deep fryer with the temperature held at 200 °C for 5 min.

Statistical design

The State of Mexico was taken as a model from the observation of the eating habits of university students to provide a product with sensory characteristics that meet the standards of quality and accessibility. A statistical-descriptive study was carried out through an analytical survey of the university population of the State of Mexico, located in the central zone of the Mexican Republic, at an altitude of 2683 m above sea level, made up of a total of 125 municipalities, has a territorial extension of 22,499.95 km^2 and a total of 16,992,418 inhabitants (INEGI, 2021), this being the state with the largest number of inhabitants in the republic. Surveying mainly autonomous universities (UAM, UAEMEX, etc.), technological institutes (UTVT, Technological Institute of Toluca, Tecnologico de Jocotitlan, etc.), and private (UNITEC, UVM, Universidad Milenium, etc.).

Description of the study

The obtaining of information was carried out through an analytical survey established by multiple-choice with the help of google forms, applied from September 6 to September

11, 2021, with an average duration of 3.5 minutes, to 137 people randomly university students of the State of Mexico with an age range of 17 to 28 years. Selecting topics related to the consumption of legumes to know the perspective of the consumer, his preference for them, and the habituality of their consumption, thus finally focusing on peanut and bean flours (Physical Environment Mexico, 2021).

Questionnaire and measurement scale

The questionnaire was divided into five sections: The first section seeks to clarify the definition of snack, as well as to know the percentage of consumption and preferences of the object of study on it. The second section is aimed at the knowledge of the participants about legumes, mainly in their importance in the diet, the frequency of their consumption, and the health benefits, including the nutrients they provide us. The third section focused on legume snacks, respondents were asked to provide information on: if they knew of any snack made from any legume flour if they had already consumed it if they were interested, and what type of legume flour snacks they would like to consume and in what form. In the fourth section he focused on the idea of the legumes selected in particular from flour, they were asked if they would be struck by any presentation of a snack based on bean and peanut flour in the market and if they considered that this snack was a good option to include it in their diet. In the fifth section, an approach was considered aimed at the packaging, portion, and cost of the product, to know the preferences and the value that the product could come to quote in the market and thus properly define the tastes of future consumers.

Method for sensory evaluation

Affective test

The evaluation was realized at the State of Mexico Universities. Sixty-eight consumers (age 18-33) assessed the snacks. They evaluated the acceptance in the categories of odor, taste, hardness, easy to break, and overall liking using a structured hedonic scale of 7 points (1=dislike a lot, 7=like a lot). Each sample was presented monadically to the consumers in a balanced complete block design.

Statistical analysis

A comparative analysis of the information obtained in the surveys was carried out, considering the sociodemographic variables of the female and male gender. The processing began with the organization of the database in Microsoft Excel. Subsequently, the values of each of the answers were calculated, and finally, the statistical software SPSS was used to identify the significant differences between the responses of the respondents ($p<0.05$), applying the non-parametric Chi-square test. The t-Student test was conducted on an affective test by XLSTAT software version 2014.5.03 (Addinsoft, Paris, France) using an alpha limit value of 0.05 for affective test.

RESULTS AND DISCUSSION

Many snacks are classified as junk food due to their low nutrient content, high preservative content, and high sodium, sugar, and/or fat (Hess *et al.*, 2016; Neder-Suárez *et al.*, 2021). Currently, most snacks available are manufactured from cereals tending to be high in fat content, rapidly digestible starch, salt, and sugars, which could be contributing to the increased prevalence of obesity and diabetes (Tas & Shah, 2021). For all the above reasons it is important to know the food habits of university students, to offer new food developments that contribute to nutrition and can be incorporated into the diet as snacks. Figure 1 shows the differences in snack consumption in the gender populations. The participants were 30% male and 70% female. In this case, the female gender presented twice as many consumption preferences compared to the male population, these being significantly different ($p<0.05$), unlike meat which does not present a difference in consumer preference between the genders. In addition, it can be observed that the female gender presents a 37% higher consumption of potato chips in comparison with the male gender, which together with the high consumption of nuts can be an indicator of the segment of the university population in which the consumption of chips-type snacks made from black bean and peanuts could be implemented.

In Germany, men judged the quantity of recommended fruit and vegetable servings to be substantially lower than women in a study of older UK individuals, aged 55-64 years (3.4 *vs.* 4.5 portions, respectively). Similarly, only 28% of guys properly knew that eating five or more servings per day is suggested, but 63% of ladies were aware of this guideline. Furthermore, just 28% of men, compared to 35% of women, were aware of the link between disease and fruit and vegetable consumption. In contrast with this study, Nutrition Report 2000 in Germany, the average meat consumption of men was 10-20 grams per day more than that of women, depending on age group (Westenhoefer, 2005).

Figure 2 shows the place where the products are purchased. Women have more preference than men to buy snacks in supermarkets and self-service stores, in markets, and prepared at home ($p<0.05$). Schlinkert *et al.*, 2020 reported that young people in the Netherlands went to snack outlets, as a result, levels of obesity and overweight increased. However, they are snacking at home (58%), at work, or at school (23%). They ate at home

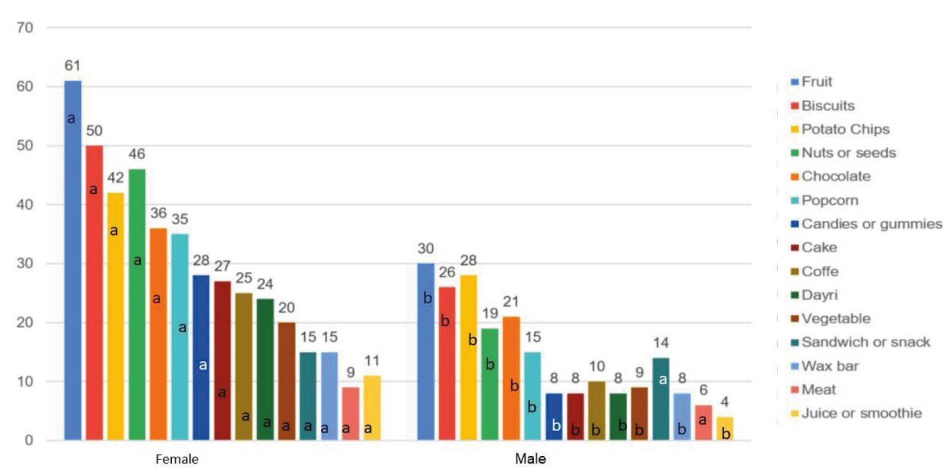

Figure 1. Frequency (%) of consumption of snacks in a population of university students in the State of Mexico. [a-b] Different letters between the same variable value indicate a significant difference between genders ($p<0.05$).

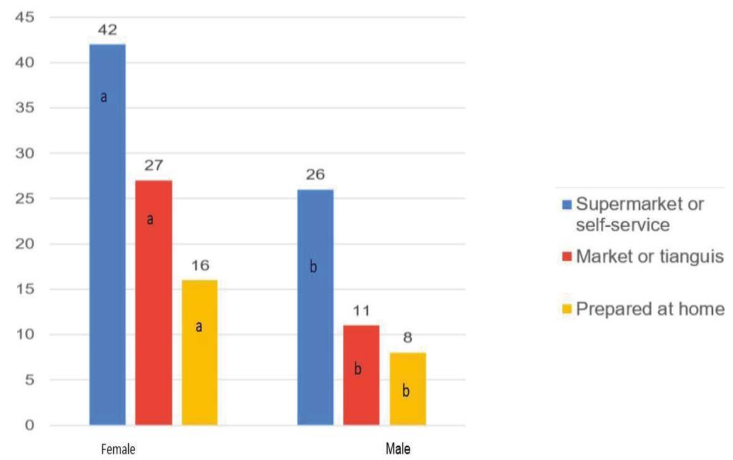

Figure 2. Frequency (%) of the place where snacks are obtained in a population of university students in the State of Mexico.
[a-b] Different letters between the same variable value indicate a significant difference between genders ($p<0.05$).

because of the price of snacks, and they prefer to buy them at local supermarkets than "on the go".

Likewise, snacks are listed as impulse products, which are frequently described as low cost, and require the consumers to use minimal cognitive effort. Because it includes automatic and habitual subconscious decisions, it was discovered that the inclination to buy on impulse is significantly linked to the habit of eating snack foods (Duarte *et al.*, 2013).

Figure 3 shows the types of flour that the population prefers in the preparation of snacks. Peanut and chickpea flour are the most preferred by the female population ($p<0.05$) unlike the male population. Subsequently, the uses of the other flours such as broad beans and lentils are lower consumption between both genders ($p>0.05$).

Figure 4 shows the types of snacks consumed. Crispy chips and churros were highly preferred by the population, with women consuming more ($p<0.05$). Subsequently, snacks

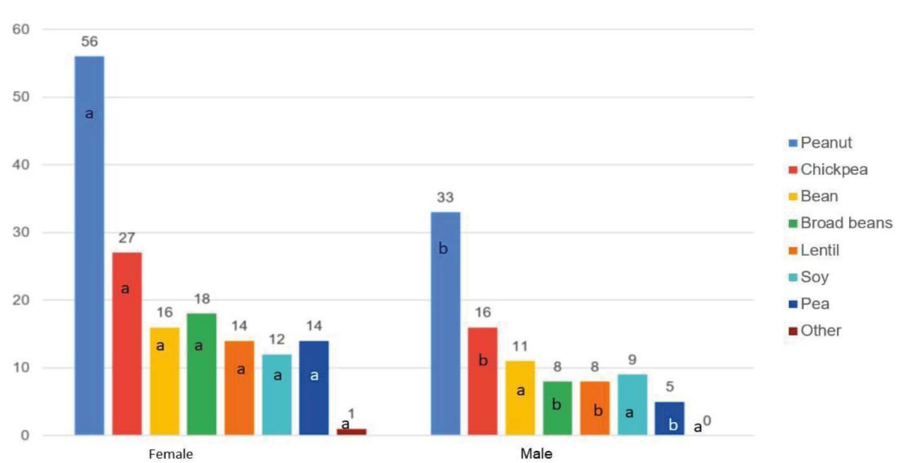

Figure 3. Frequency (%) of the type of flour preferred in snacks in a population of university students in the State of Mexico.
[a-b] Different letters between the same variable value indicate a significant difference between genders ($p<0.05$).

made from wheat flour and others had lower consumption but were similar between genders ($p>0.05$). Duarte *et al.*, 2013 reported that because several studies have found a link between snack food consumption and the overweight and obesity problems of young people, public health policies and campaigns will be aimed at the development of healthy snacks.

Figure 5 shows the preference of the place in the consumption of snacks. Women prefer to consume these products more frequently in their homes compared to men ($p<0.05$). While, in other places such as the university, work, street, and other places, consumption was lower in both genders and without significant differences ($p>0.05$).

Figure 6 shows the nutritional characteristics of the snacks. Women prefer products with higher content of vitamins, minerals, and fiber than men ($p<0.05$). In the case of protein, calories, fat, and sodium contents, there were no significant differences between genders ($p>0.05$).

Similarly, Westenhoefer (2005) reported that gender-dependent differences in food choice, women generally show a healthier pattern of food choice. For example, the

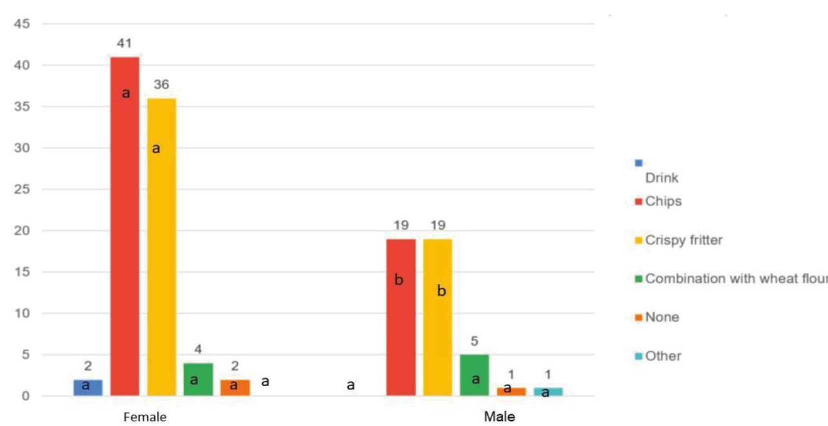

Figure 4. Frequency (%) of the type of consumption of snacks in a population of university students in the State of Mexico.
[a-b] Different letters between the same variable value indicate a significant difference between genders ($p<0.05$).

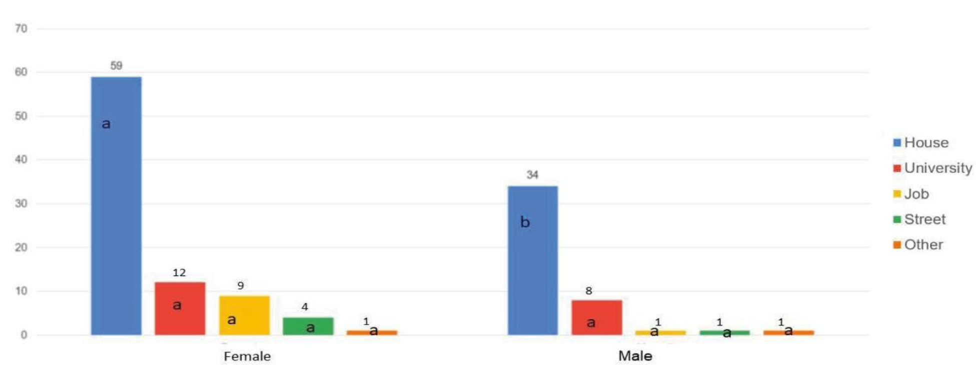

Figure 5. Frequency (%) of the preference of the place for the consumption of snacks in a population of university students in the State of Mexico.
[a-b] Different letters between the same variable value indicate a significant difference between genders ($p<0.05$).

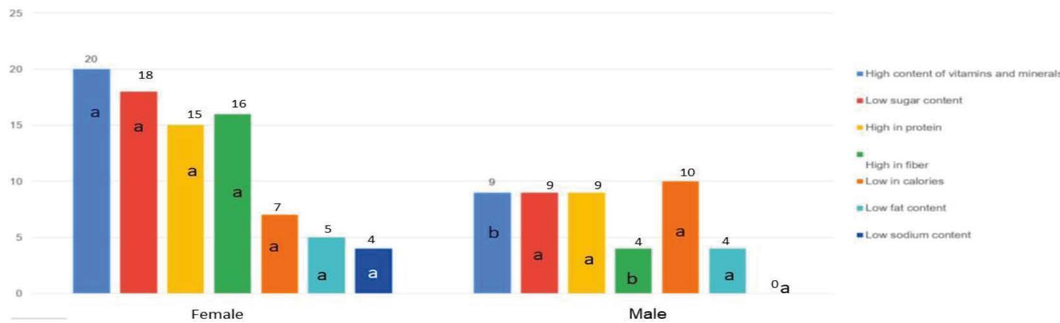

Figure 6. Frequency (%) of the preference of the nutritional characteristics of snacks preferred by the population of university students in the State of Mexico.
[a-b] Different letters between the same value of the variable indicate a significant difference between genders ($p<0.05$)

International Health and Behavior Survey (IHBS) applied a self-report, questionnaire-based study that looked at a variety of health behaviors in a total of 19,298 university students from 23 different nations. Women avoid high-fat foods, consume high-fiber foods, eat fruit every day, and never add salt to their meals compared to men. Likewise, consumers purchasing snack foods evaluated health/nutrition as the second most important attribute after taste/flavor in New Zealand (Kahiya *et al.*, 2013). In the end, because gender has an inextricable link with purchasing decisions, it must be analyzed separately for each product and company (Zoi, 2017).

Affective test

There was no significant difference ($p>0.05$) between the samples prepared with beans and peanuts for all categories. The rating of the two samples was "Like" in all categories (Figure 7).

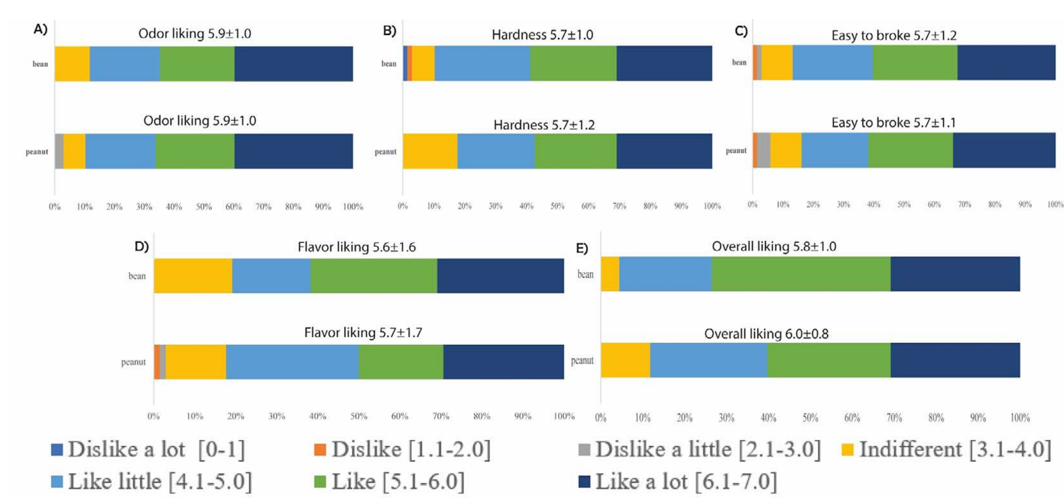

Figure 7. Frequency of acceptance of odor (A), hardness (B), easy to break (C), flavor (D), and overall liking (E) (media±std). No significant difference ($p>0.05$) between bean and peanut snacks.

CONCLUSION

The consumption of legumes is an important basis for a healthy diet. To know the sensory perspectives of university students could provide important information to develop healthier and easier-to-eat snacks. The chips-type snacks made with PF and BbF could be a healthy alternative for the younger consumers, also it kinds of products have good acceptability between them. However more studies about the characterization of the snack are required, also a mix of both legumes could be realized to obtain an add value product.

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Reproductive and morphological phenology of eight Mexican cacao clones (*Theobroma cacao* L.)

Rivera-Torrez, Diana L.[1]**; Lagunes-Gálvez, Laura M.**[1]**; Azpeitia-Morales A.**[2]**; García- Alamilla, Pedro**[1*]

[1] División Académica de Ciencias Agropecuarias, Universidad Juárez Autónoma de Tabasco. Carretera Villahermosa-Teapa km. 25, La Huasteca, Centro, Tabasco Villahermosa, Tabasco, México. C.P. 86280.
[2] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Campo Experimental Huimanguillo. Carretera Huimanguillo-Cárdenas Km 1. Tabasco, México. C.P. 86400.
* Correspondence: pedro.garciaa@ujat.mx

ABSTRACT

Objective: To describe the dynamics of floral biology and fruit development until physiological maturity, by performing a qualitative and quantitative characterization in eight Mexican cacao clones (*Theobroma cacao* L.), called: Caehui, Chak, Canek, Chibolon, Supremo, Tabscoob, K'in and Olmeca.

Design/methodology/approach: In the phenological study, five trees were taken into account and five flower buds per tree were randomly identified (n=225). Twenty-one (21) qualitative and quantitative morphological descriptors were evaluated in fruits and grains. Descriptive statistics were established and a principal component analysis was applied to the quantitative descriptors.

Results: The Chak clone presented the highest value for length and diameter of flowers buds in reproductive phenology, with 7.4 mm and 4.2 mm; the K'in clone stood out in fruits, with 252 mm length; and Supremo in fruit diameter, with 102 mm. Significant differences were found in the morphological variables evaluated, in addition to significant positive correlation ($p<0.05$ and $p<0.001$) between most of the variables. The first two main components described 62.5% of the total variation.

Findings/conclusions: The differences in measurements (length and diameter) of the flower buds are attributable to the genetic constitution; however, knowing the opening times allows us to have an exact reference in production levels. The parameters that contributed most to the variability observed were weight and diameter of the fruit, length and width of the grain, thickness of the exocarp, and weight of grains per pod.

Keywords: cacao beans, clones, growth dynamics, descriptors.

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INTRODUCTION

Genetic diversity in cacao contributes to the sustainability of the global economy of cacao, with particular interests that range from production, resistance, nutraceutical aims, product elaboration, to the selection of cacao that fulfills quality requirements (Aikpokpodion, 2010). Morphology and geographical origin have been defining parameters in the description of genetic groups of cacao, where phenotypical differences

Image by David Greenwood-Haigh at Pixabay due to genetic variability are observed, as well as qualitative and quantitative differences in the indicators of cacao fruits. Studies report morphological variations in fruits of Criollo, hybrid cacaos and international reference material, showing the heterogeneity between plantations. The morphological characteristics of the pods, seeds and flowers have also been used to evaluate the relationships between cacao genotypes (Ramírez *et al.*, 2018; Ph *et al.*, 1999). A study conducted by López-Hernández *et al.* (2018) reported variability in length and diameter in buds and fruits of clones, and Aikpokpodion (2010) reported 43.6% of the total variation observed in qualitative and quantitative ranges of fruits. On the other hand, Bekele *et al.* (2006) obtained significant variations in morphology in studies about cacao accessions. Part of the descriptions of differentiation and characterization stems from the reproductive phenology in cacao until maturity, the varieties tend to have different characteristics in their flowering, appearance of flower buds and fructification (Santos *et al.*, 2012; Tabla & Vargas, 2004). These descriptions provide growth patterns in an objective and unambiguous manner, as indicated by Melgarejo (2015), mentioning that with bud measurements the trends in the shapes of fruits can be known according to the length and diameter ratio. Therefore, the use of tools for qualitative and quantitative descriptors allows obtaining elements that highlight the potential of new clones in relation to cacao with quality, productivity and yield. Anita-Sari, I., & Susilo (2015) highlighted the importance of the growth cycle with the yield and formation of fruits, and in their studies, Marcano *et al.* (2009) determined the mixture mapping of the entire genome of cacao groups for yield factors and morphological traits, defining the characterization of the first link in the amplitude of the variation and the trend towards phenotypical characteristics. Therefore, the research objective was to describe the floral biology and fruit development until physiological maturity, in addition to their qualitative and quantitative characterization in eight new clones generated in the germplasm garden of the INIFAP Experimental Field in Huimanguillo, Tabasco, Mexico.

MATERIALS AND METHODS

Variables for flower buds and fruits: Eight clones were evaluated for this study: Caehui (UF 613×IMC 67), Canek (RIM 75×SPA 9), Chak (RIM 76A×EET 48), Chibolón (UF 613×Pound 7), K'in (CC 226×IMC 67), Olmeca (RIM 76A×EET 400), Tabscoob (RIM 76A×EET 48×PA 169) and Supremo (UF 613×IMC 67), in addition to a control called Carmelo (SNICS 1036), in the National Institute for Forestry, Agriculgture and Livestock Research (*Instituto Nacional de Investigaciones Forestales Agricolas y Pecuarias*, INIFAP) at the experimental field in Huimanguillo, Tabasco, Mexico (17° 47' 07.9" N and 92° 57' 20.0" W), inside the cacao germplasm garden (INEGI, 2018). Five trees were selected and five flower buds were selected randomly without considering position on the tree (n=225 flower buds). The measurements were made with a digital Vernier (ISIZE, 0-150 mm, Mexico) on the length and diameter of the flower bud without considering the stem, between 30 and 35 days since the flower bud emerges until the opening happens. Starting with the formation of the fruit, the morphology of the fruit was evaluated every 15 days until reaching physiological maturity (Enríquez, 2004).

Morphological variables: Nine qualitative morphological variables were analyzed: Shape of the pod (SP), basal construction (BC), shape of the apex (SA), surface of the pod (S), depth of the furrows (DF), color of the pulp (CP), shape of the grain (SG), color of the cotyledon (CC), color of the fruit; and 12 quantitative variables: fruit diameter (FD), fruit length (FL), fruit length/diameter ratio (FL/FD), fruit weight (FW), exocarp thickness (ET), grain number (GN), grain weight per pod (GWP), grain length (GL), grain width (GW), grain length/width ratio (GL/GW), grain thickness (GT), average grain weight (AW), according to the International Union for the Protection of New Varieties of Plants *Theobroma cacao* L. (UPOV 2011). The information was analyzed by defining descriptive statistics, principle components analysis (PCA), and Pearson's correlation, using the statistical package Metaboanalyst 5.0. and Infostat-Profesional, version 1.1 (2020).

RESULTS AND DISCUSSION

Flower buds

Figure 1 shows the evolution of the flower bud growth until its opening with relation to the bud total length and diameter, starting for 7 days after installation (dai) and lasting until 30 to 35 days, similar to what was reported by López *et al.* (2018). Caehui obtained a record of 42 days, which was higher than the other clones, associated to intrinsic, genetic and physiological factors. The clones that present the highest total length ± standard deviation of the bud were Chak with 7.4±0.01 mm, Canek and Caehui with 7.3±0.20 mm, respectively, and the lowest total length was seen in Chibolon with 6.2±0.10 mm. The other clones remained at between 6.2 and 7.3 mm. In the variable diameter, the clones that reached the highest value were Chak with 4.2±0.20 mm, Caehui and Tabscoob, both with 4.1±0.13 mm. The clones K'in and Canek were the ones that presented the smallest diameter with 3.5±0.20 mm each, the other clones and the control were found between 3.2 and 4.2 mm. Castro *et al.* (2017) report an initial value of 4±1 mm and final value of 15±1 mm, data that are apparently higher than those reported for this study, although they consider the stem within the measurement of the flower buds.

Figure 1 does not show a sigmoidal pattern as reported by López *et al.* (2018); however, a linear behavior was seen for longitudinal growth since day 7 achieving 42% of the final size, and prolonged until day 21 with 75% of its final size. For the variable diameter, 52% was achieved in the first seven days while 77% of this variable was reached starting on day 21.

Growth in cacao fruits: Changes in fruit length and diameter (Figure 2) were described in this stage, considering the measurement unit from the moment when it reached the critical point of 15 days after the start of pollination.

The highest value in length was K'in with 252±3.54 mm and Olmeca with 220±0.70 mm, in a period of 195 days and 165 days, respectively. Meanwhile, Caehui with 103±3.74 mm presented the lowest length in 120 days. For the final diameter of the fruits with largest diameters, it was between 102±4.9 mm in Supremo with 150 days, followed by Tabscoob with 101.2±1.14 mm with 195 days.

The result of the lowest diameter at the end of development was shown by Caehui with 38.6±5.98 mm in 120 days. Figure 2 shows that the growth speed presented an inflection

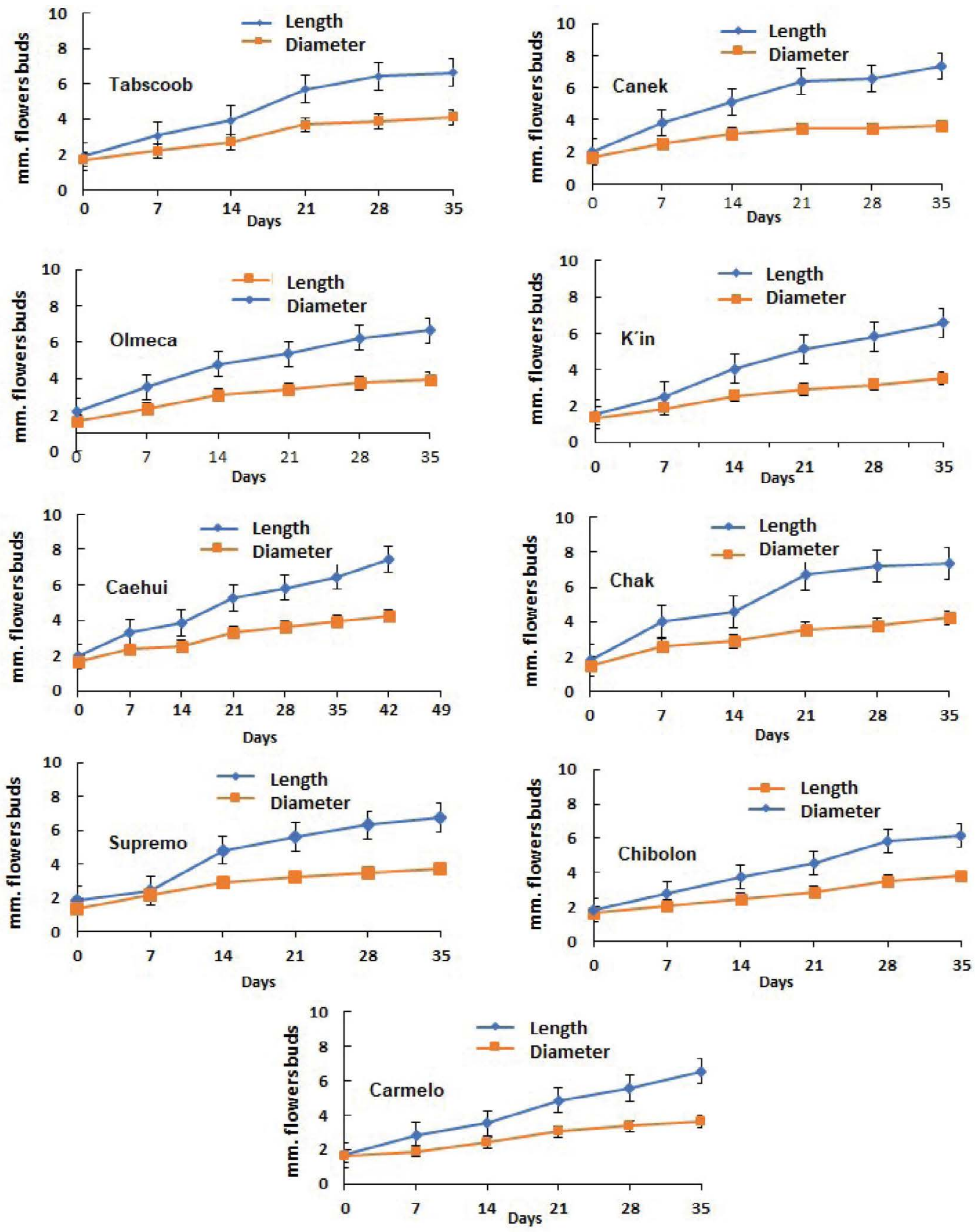

Figure 1. Evolution of phenological growth of diameter and total flower bud lenght in eight cocoa clones and Carmelo control. The mean values and the vertical line indicate ± standard error (SE).

point since day 90, with fruits in this stage reaching an increase in length and diameter of 38%, which represents the change in linear trend toward a non-linear increase. Of the fruit growth, 70% was achieved in 120 days, period at which the growth speed is slow since the cell division and elongation processes stop, causing the change in color of the fruits (López *et al.*, 2018; Enríquez, 1985). Starting at 165 days, it is the last phase of development, without apparent changes, which is associated to the maturation of the fruit.

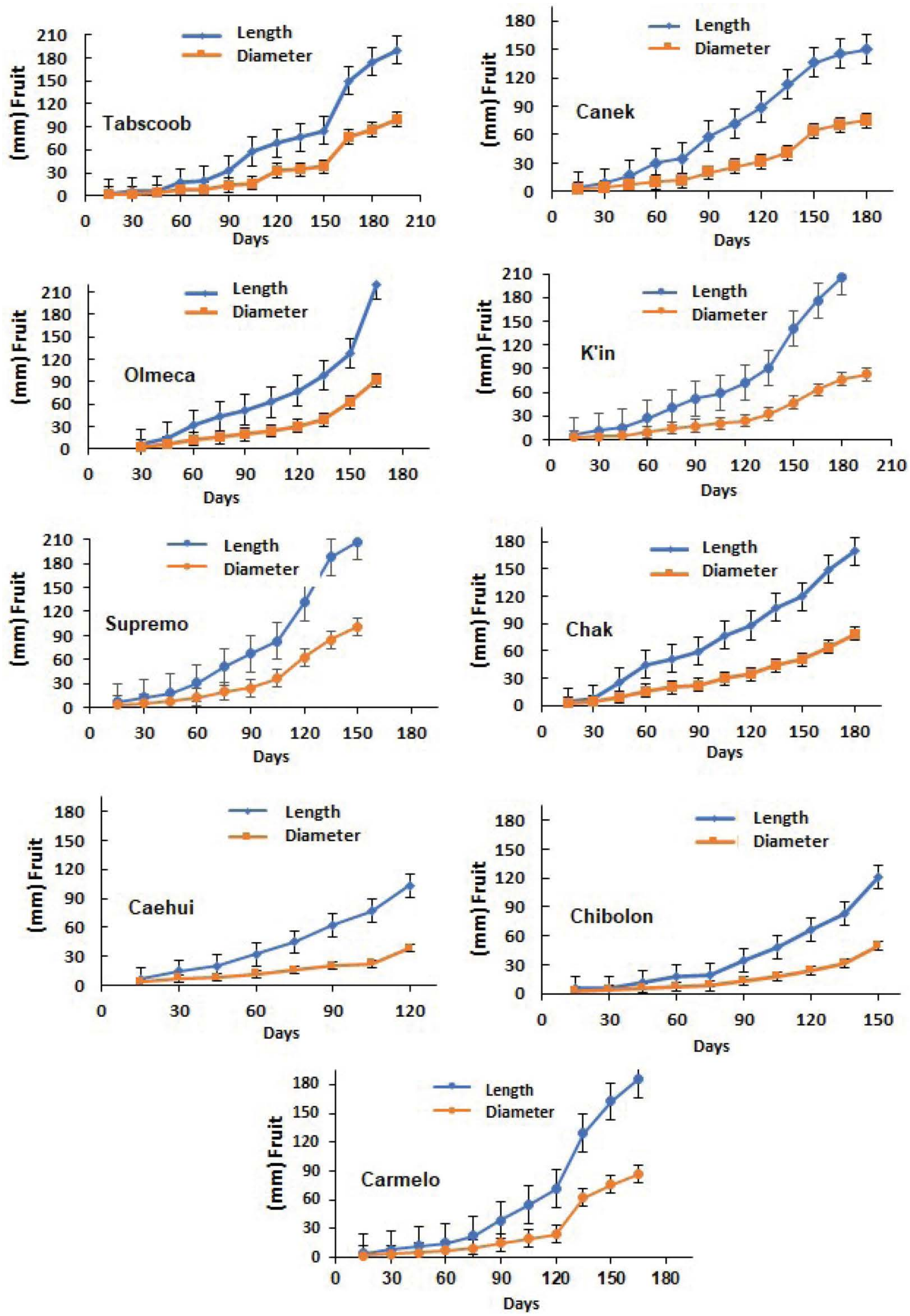

Figure 2. Evolution of phenological growth of diameter and total friut lenght in eight cocoa clones and Carmelo control. The mean values and the vertical line indicate ± standard error (SE).

Qualitative morphological characterization in the fruits

The physical characteristics in fruits of the clones were recorded in Table 1. The analysis result showed that the elliptical pods (44.4%) dominated as morphological characteristic of the fruit shape, followed by oblong (33.3%), which was similar to what was reported by Castillo (2018), who found mostly elliptical shape which refers to these characteristics being of Creole type. In the case of the basal construction, the absent state predominated with 55.5%, followed by the weak and moderate state (22.2%). Another predominant characteristic in fruits for this study was the surface, presenting smooth or slightly rough with greater frequency, with global percentage of 77.7%. In this regard, Graziani *et al.* (2002) found that sharp tips, absent basal construction and intermediate roughness predominate in the Creole-type cacao, while forastero types present melon

Table 1. Frecuency of qualitativa variables in fruits of cocoa clones.

Variables	State	% global
1) Fruit: shape	1=ovate 2=circular 3=elliptic 4=oblong 5= obovate	11.11 0 44.44 33.33 11.11
2) Fruit: basal constriction	1=absent 2=weak 3=moderate 4=strong	55.55 22.22 22.22 0
3) Fruit: shape of apex	1=waisted 2=acute 3=obtuse 4=rounded	11.11 44.44 44.44 0
4) Fruit: Surface	1=smooth or slightly 2=moderately rough 3= very rough	77.77 22.22 0
5) Fruit: Depth between ridges	1=absent or very shallow 2=shallow 3=medium 4=deep	33.33 66.66 0 0
6) Fruit: Color of pulp	1=white 2=ligth cream 3=dark cream	11.11 66.66 22.22
7) Seed: shape in longitudinal section	1=oblong 2=elliptic 3=ovate	33.33 11.11 44.44
8) Seed: cotyledon color	1=white 2=cream 2=pink 3=dark red 4=dark purple	11.11 11.11 11.11 33.33 33.33
9) Fruit: color	1=yellow 2=yellow/green 3=yellow/orange 4=orange/purple	22.22 44.44 22.22 11.11

shape, obtuse tip, without strangling and without roughness. In another context, the fruit color reached a different coloration when it was found in physiological maturity; in this case 44.4% corresponded to yellow/green tonalities, 22.22% to yellow and yellow/orange colors. In this last morphological characteristic mentioned, Bartley (2005) revealed that the red fruit color could be representative in face of the Creole group and as a result of descendants in this group having inherited this trait. In another study, Ha *et al.* (2016) have indicated the presence of orange tones for Creole cacao, while yellow tones indicate the presence of forasterio and trinitario cacao. Our study showed that the depth of the furrows corresponded to 66.6% in the variable low depth, and some studies indicate that deep furrows are characteristic of Creole cacao (Chacón *et al.*, 2011). With 66.6% clones present medium exocarp thickness, the same percentage corresponds to the variable coloring in the pulp, with light cream color standing out. The characteristics of exocarp thickness suggest the presence of hybridization by populations of the trinitario complex, a characteristic of heterogeneity in the characteristics of the fruits (Braudeau, 1970). When it comes to the color of cotyledons, violet and purple were the dominant ones (33.33%). The color has been a distinctive trait to differentiate cultivars, highlighting that white cotyledons are associated to Creole cacao (Ramos-Ospino *et al.*, 2020). The shape in longitudinal section of the seed represented 44.44% in the oval shape.

Quantitative morphological characterization in the fruits

The clones and Carmelo were compared in terms of the morphological traits of fruit and seed of eleven quantitative traits. The variable NG showed the lowest coefficient of variation (4.52%), and the variable FW presented the highest coefficient of variation (22.17%), in addition to a high mean in comparison to the other variables studied (Table 2). In a similar study, Ramírez-Guillermo *et al.* (2018) mentioned as results intervals of broad

Table 2. Statistical parameters of 12 morphological quantitative variables evaluated in cocoa clones and Carmelo control.

Variables	Means	SD	Min.	Max.	C.V.
Fruit diameter (mm)	85.14	4.93	77.10	90.00	5.79
Fruit length (mm)	168.70	15.93	146.43	196.00	9.44
Fruit length/diameter ratio	2.03	0.23	1.63	2.35	11.19
Fruit weight (g)	574.45	127.37	403.63	840.59	22.17
Fruit exocarp thickness (mm)	13.62	1.37	11.16	15.00	10.06
Fruit number seeds	40.45	1.83	37.52	42.95	4.52
Seed weight per fruit (g)	140.75	25.78	108.27	191.07	18.32
Seed length (mm)	23.16	1.68	19.84	25.57	7.28
Seed width (mm)	13.03	1.16	10.99	14.44	8.90
Seed length/width ratio	1.79	0.09	1.66	1.99	5.09
Seed thicknees (mm)	8.51	0.50	7.51	9.20	5.88
Average seed weight	1.41	0.37	0.66	2.63	0.26

SD=Standard Deviation; CV=Coefficient of Variation (%).

variation, especially for FW. The length/diameter ratio in grain as in fruit presented lower values in terms of mean, deviation, minimums, and maximums.

Correlation of the morphological variables in cacao fruits

Table 3 shows the positive and negative correlations with significant character ($p<0.05$ and $p<0.001$). The fruit weight (FW) showed a significant positive correlation ($p<0.05$ and $p<0.001$) with most of the morphological variables with the exception of the (NG), (GL/GD) and (GT). One of the most important aspects that was found is the highly significant positive relation ($p<0.001$) between (FW) and (GW). At the same time, grain weight (GW) showed that only two variables were not significant (NG and GT) and the others showed a positive correlation ($p<0.05$ and $p<0.001$), with the exception of the GL/GD ratio which was negative. In a study conducted by Vázquez and García (2005), in a cacao germplasm bank with 20 cacao genotypes of different genetic origins found that the coefficient of correlation between the pod weight and the number of seeds was non-significant; while the correlation between the weight of the pod and the fresh weight of the seed was positive and highly significant, which agrees with our results.

Principal Components Analysis

The principal components analysis (PCA) revealed that the first axis of the CP1 is composed mainly by 37.6% of the variation, showing it is composed primarily by the geometrical traits both of the fruit and of the cotyledon: length, diameter, fruit weight and grain weight per pod correlated positively. The second axis of the CP2 represented 24.6% of the total variation, highlighting some qualitative traits, among them: surface, fruit color, shape of the cotyledon, fruit and tip, and basal construction. Figure 3 shows the relative

Table 3. Correlation coefficients of morphological variables of eight cocoa clones and the Carmelo control from INIFAP-Huimanguillo germoplasm garden.

	NG	GL/GW	FL/FD	GT (mm)	GL (mm)	GW (mm)	AW (g)	FD (mm)	ET (mm)	GWP (g)	FL (mm)	FW (g)
NG	1											
GL/GW	−0.0370	1										
FL/FD	−0.0616	−0.2430**	1									
GT (mm)	−0.1009	−0.0720	0.0277	1								
GL (mm)	−0.1274	0.3586**	−0.0868	0.0188	1							
GW (mm)	−0.0908	−0.4690**	0.1204	0.0548	0.6502**	1						
AW (g)	−0.0746	−0.1731*	0.1924**	0.1196	0.3081*	0.4437**	1					
FD (mm)	0.0645	−0.0129	−0.4216**	0.0306	0.1839*	0.1846*	0.1382*	1				
ET (mm)	−0.0629	−0.1464*	−0.0558	0.0641	0.1732**	0.2854**	0.2061*	0.5083**	1			
GWP (g)	0.1331	−0.2856**	0.1825*	0.0943	0.1620*	0.3853**	0.3592**	0.2847**	0.2809**	1		
FL (mm)	−0.0463	−0.2639**	0.6108**	0.0829	0.0267	0.2388**	0.3197**	0.4056**	0.3065**	0.4213**	1	
FW (g)	0.0605	−0.1276	0.2137*	0.0606	0.1829*	0.2901**	0.3364**	0.5029**	0.5246**	0.3787**	0.5798**	1

NG: Grain number; GL/GW: grain length/width ratio; FL/FD: Fruit length/diameter ratio; GT: grain thickness; GL: grain length; GW: grain width; AW: average grain weight; FD: fruit diameter; ET: exocarp thickness; GWP: grain weight per pod; FL: fruit length; FW: fruit weight. * ($p<0.05$), ** ($p<0.001$).

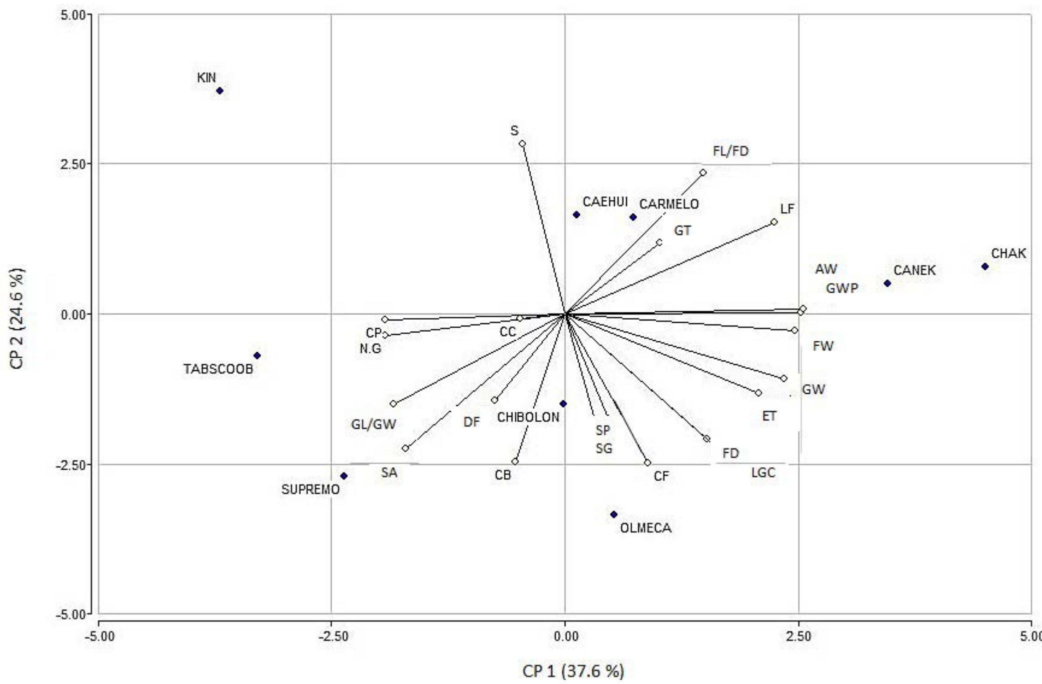

Figure 3. Dispersion of qualitative and quantitative morphological variables on the principal component axes (CP1 and CP2) accounting for 62.5% of the total variation. NG: grain number; GL/GW: grain length/width ratio; FL/FD: fruit length/diameter ratio; GT: grain thickness; GL: grain length; GW: grain width; AW: average grain weight; FD: fruit diameter; ET: exocarp thickness; GWP: grain weight per pod; FL: fruit length; FW: fruit weight; SP: Shape of the pod; BC: basal constriction; SA: shape of the apex; S: surface of the pod; DF: depth of the furrows; CF: color of the fruit; CP: color of the pulp; CC: color of the cotyledon; SG: shape of the grain

association between variables, in function of the principal coordinates that accounted for 62.5% of the total variation.

CONCLUSIONS

The differences in measurements (length and diameter) of the flower buds are attributable to the different genetic constitution of the clones; however, knowing the opening times of these allows having an exact reference in production levels. The first two principal components (PC) in the morphological analysis described 62.5% of the total variation. The parameters that contributed most to the variability observed were FW, FD, GL, GD, ET and GWP. The study shows that the clones presented morphological variation for the characteristics of the fruit, and with that it would be expected that the physicochemical characteristics give different quality characteristics. The study revealed specific characteristics of the clones that can be used as selection criteria to improve the yield and quality indicators.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Cassava-based (*Manihot esculenta* Crantz) fermented energy-protein food for bovines

Ramos-Juárez, Jesús A.[1*]; Reyes-Cruz, Luis A.[1]; Bucio-Galindo, Adolfo[1]; Zaldívar-Cruz, Juan M.[1]; López-López, Rutilo[2†]

[1] Colegio de Postgraduados, Campus Tabasco, Carretera Libre Cárdenas-Coatzacoalcos km 21, Poblado C-27, Cárdenas, Tabasco, México, C. P. 86500.
[2] Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, Campo Experimental Huimanguillo, Carretera Federal Huimanguillo-Cárdenas km 1, Huimanguillo, Tabasco, México, C, P. 86600.
* Correspondence: ramosj@colpos.mx

ABSTRACT
Objective: To assess the effect of different yeast levels and fermentation times on the chemical, fermentative, and microbiological composition of cassava in order to produce a cassava-based fermented food.
Design/Methodology/Approach: We used a completely randomized design with a factorial arrangement, three yeast inoculum levels (0, 5, and 10%), and five fermentation times (0, 1, 2, 3, and 4 days).
Results: We found significant interaction of the studied factors with the pH and crude protein (CP) variables. The highest pH values were obtained adding 10% yeast inoculum (YI) (8.67). CP values of 16.55% were found. No differences caused by the studied inoculum levels and fermentation times were found in true protein (TP) and the *in situ* degradation of dry matter (IDDM). TP and IDDM had 8% and 80% values, respectively.
Study Limitations/Implications: A behavior test with bovines must be conducted to demonstrate the potential of cassava-based fermented foods in meat and/or milk production.
Findings/Conclusions: The yeast inoculum and the fermentation days did not increase the TP in the cassava-based fermented food.

Key words: Protein, Tuber, Solid state fermentation.

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INTRODUCTION

Grasses are the basis of the diet in bovine production systems. However, since grass production and quality present seasonal variations, supplements are needed to complement its biomass and nutrient deficit and, consequently, to increase the production of meat and milk (Ramos *et al*., 2018). The use of commercial concentrates is not always an option for small and midsized producers due to their high market cost. Therefore, it is necessary to search for alternative supplements based on raw materials that farmers can produce in their own farms and then subject them to a protein enrichment process.

Image by Brett Hondow at Pixabay

Cassava is a tropical crop used as food for both humans and animals. Its root (tuber) contains approximately 65% moisture. To use it as food, cassava is cut in 1-2 cm thick slices and then laid out in the sun to reduce its moisture content to 15 or 18%. The tuber has a significant content of high-digestibility starch (60-72%) and a low protein content (2.4%). Therefore, its use in supplements requires high quality protein ingredients (De Blas *et al.*, 2010), which are the most expensive ingredients in livestock diets.

Solid state fermentation (SSF) is a biotechnological process used to enrich fibrous products or by-products with low protein contents (Ramos *et al.*, 2018). Microorganisms (yeasts) are used to increase microbial biomass, which in turn increases the protein content of the fermented material (Jiménez-Alfaro *et al.*, 2020).

The objective of this work was to evaluate the effect of different yeast levels and fermentation times on the chemical, fermentative, and microbiological composition of cassava in order to produce a cassava-based fermented food.

MATERIALS AND METHODS

Site of the experiment

The experiment was conducted at the Colegio de Postgraduados, Campus Tabasco, Cárdenas municipality, Tabasco, Mexico (18° 00’ N, 93° 30’ W, at an altitude of 12 m).

Treatments and experimental design

We used a completely randomized experimental design with a 3×5 factorial arrangement, three levels of yeast inoculum (0, 5, and 10%), and five fermentation times (0, 1, 2, 3, and 4 days), with a total of 15 treatments and four replications per treatment (60 experimental units, Roux bottles).

Preparation of the yeast inoculum

The yeast inoculum (YI) was prepared through the liquid state fermentation process, using molasses (15%), soy paste (4%), polished rice (4%), mineral salts (0.5%), magnesium sulfate (0.32%), urea (0.48%), yeast (0.5%), and water (75.2%). The ingredients were mixed in a 2-L beaker that was shaken in a DBO 200 incubator (Novatech®, Mexico) for 5 minutes, three times a day for three days, at a temperature of 30 °C.

Preparation of the cassava-based food for fermentation

We used sugarcane, which we cut 24h before use (Elías *et al.*, 1990) and let stand in the shade. Afterwards, we grinded it in a PFA 3000 forage chopper (8 HP Power Cat, Mexico). We grinded sweet cassava bought in a public market in a LI-12A industrial blender (TAPISA®, Mexico). We mixed a 250-g sample according to the percentages shown in Table 1 and added the corresponding YI to each treatment (0, 5, and 10%). We determined the YI moisture and added water to adjust the initial dry matter (DM) of the feed to be fermented.

We then put the mix in 1-L Roux bottles (Pyrex®, USA) —whose mouths were covered with cotton to produce aerobic fermentation— and then placed the bottles in a DBO 200 incubator (Novatech®, México) at a temperature of 30 °C. Mineral salts (LAPISA®,

Table 1. Inclusion percentage of the ingredients to be fermented.

Ingredients	Content (%)
Cassava meal	62.2
Soybean meal	4.0
Urea	1.5
Sugarcane	30.0
Magnesium sulphate	0.3
Mineral salts	2.0

México) contained 12% P, 13% Ca, 15.6% Cl, 10.4% Na, 0.6% Mg, 0.3% S, 0.12% Zn, 0.12% Mn, 0.03% Cu, 50% Co, and 3.0% Se.

Fermentative analyses

After the different fermentation times (0, 1, 2, 3, and 4 d) had ended, the entire content of the Roux bottles was collected on a crystal plate and homogenized. We took a 10 g sample, to which we added 90 mL of distilled water; subsequently, the whole sample was shaken in an orbital shaker (VWR DS-500E®, USA) for 30 min. We immediately measured the pH with a potentiometer (Denver Instrument®, USA) and the °Brix in a 00700 handheld refractometer (ATAGO®, Japan).

Chemical analyses

We put the solute in a paper bag, weighed it, and then placed it in a forced air stove at 62 °C, until it reached a constant weight. Subsequently, we grinded it in a Model 4 mill (Thomas Wiley®, Germany) with a 2-mm sieve, in order to conduct the following analyses: moisture and crude protein (CP), following AOAC (2016); true protein (TP), according to Meir (1986); neutral detergent fiber (NDF) and acid detergent fiber (ADF), following Lourenco *et al.* (2017); and *in situ* degradation of dry matter (IDDM), according to Haile *et al.* (2017). We subtracted the moisture percentage from 100 to calculate dry matter (DM).

Microbiological analyses

We took a 10 g sample, mixed it with 90 mL peptone water (Official Mexican Standard NOM-110-SSA1-1994, 1994), and then shook it in an orbital shaker (VWR DS-500E®, USA) for 30 min. Afterwards, we filtered the samples with sterile gauze pads and made dilutions with 1 mL of the filtered material. The potato dextrose agar (PDA) medium was used to quantify the yeast, while the Man Rogosa and Sharpe (MRS) medium was used to quantify the lactic bacteria, with 10^{-5}, 10^{-6}, and 10^{-7} dilutions in duplicate, using the pour plate method. According to the fermentation days, 90×15 mm sterile Petri dishes were incubated in inverted position at 30 °C.

Statistical analysis

The resulting data were analyzed with ANOVA statistical tests in order to determine differences between treatments. Means were compared using Tukey's test with the SAS software (2017), version 9.4. The separate effect of the studied factors was assessed with SAS's Slice tool when variable interaction was found.

RESULTS AND DISCUSSION

Fermentative analyses

Significant interaction was recorded between the studied factors in the pH and °Brix variables. The highest pH values were reached when 10% YI was added on fermentation days 0, 1, 2, and 3 (Figure 1).

The pH is decisive for yeast growth and microorganism development in SSF. In this work, pH values ranged from 6.14, with the addition of 0% YI, to 8.67, with the addition of 10% YI. A pH of 4.5 to 6.5 is considered optimal for yeast growth in the SSF process. Nevertheless, Elías *et al.* (1990) found that the optimal pH for yeast growth in the SSF-induced enrichment of sugarcane was 3.5 to 6.

No effect was found on the °Brix at the studied YI levels on fermentation days 0 and 3; however, the lowest °Brix values were found when 5 and 10% YI were added on fermentation days 1 and 2, with no difference between them (Figure 2).

The reduction in sugar concentration on fermentation days 1 and 2 (when 5 and 10% YI were added) could have indicated that yeasts were using sugar and that further growth was to be expected. However, this did not happen, probably as a result of the high pH value.

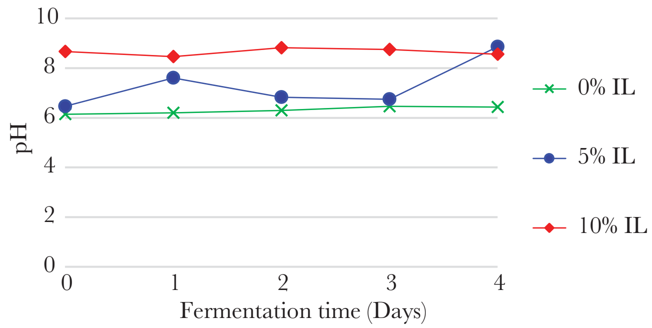

Figure 1. Effect of yeast inoculum (YI) levels and fermentation days on the pH of the fermented food.

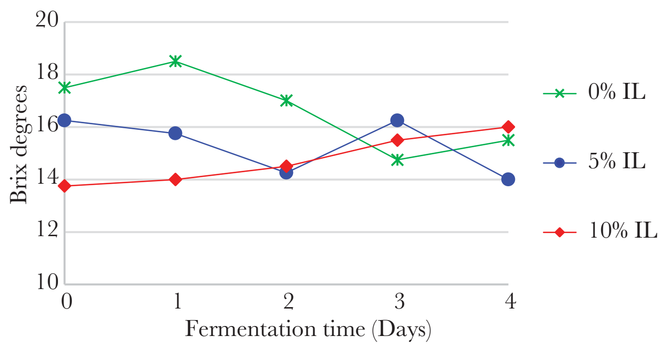

Figure 2. Effect of yeast inoculum (YI) levels and fermentation days on the °Brix of the fermented food.

Chemical analyses

We determined that the studied factors interacted with the CP variable. There were differences only on days 1 and 2. The highest CP values on day 1 were obtained with the addition of 0 and 10% YI, with no statistical difference between the two percentages. The highest CP values on day 3 were obtained with the addition of 5 and 10% YI, with no difference between them (Figure 3).

Cassava has a 2.4% CP concentration. In this work, values of up to 16.56% were reached at the end of the fermentation process; these values are within the range used in commercial supplements for bovines in the growth stage. This increase in CP is caused by the addition of urea (1.5%) and soy paste (4%). Similar data were reported for pineapple silages, using a yeast inoculum: CP levels increased from 8.7% to 18.5% (Rodríguez *et al.*, 2014).

Urea is the cheapest source of non-protein nitrogen (NPN) used in ruminant diets; if there is an available energy source, it can be used by rumen bacteria to synthetize amino acids. However, if it is not used correctly, animals run the risk of suffering intoxication, as a consequence of the fast transformation of urea into ammonia (NH_3), which increases the rumen pH and the permeability of the rumen walls to non-ionized NH_3 (McDonald *et al.*, 2013). Just as in the case of this study, an advantage of feed produced by SSF is the absence of urea-related intoxication risks when the food is eaten fresh, because, at the end of the SSF process, the NPN of urea becomes ionized ammonium $\left(NH_4^+\right)$, a substrate that can be used by rumen microorganisms and to which rumen walls are less permeable. In addition, voluntary consumption of the fermented product is slow (Ramos *et al.*, 2016).

Due to the initial addition of water, no differences were found in the DM content of the cassava-based fermented food between the different inoculum levels or the fermentation times (Table 2). DM varied between 33 and 34%; these data are similar to those reported by Rodríguez *et al.* (2014), who added molasses, urea, and a yeast inoculum to pineapple silages.

No differences resulting from the inoculum levels or fermentation times were found in TP (Table 2). TP can provide an indirect way to measure microbial growth in SSF processes, because the established microbiota transforms the NPN of urea into protein nitrogen (PN) (Ramos *et al.*, 2006). In average, the TP percentage of the fermented foods of

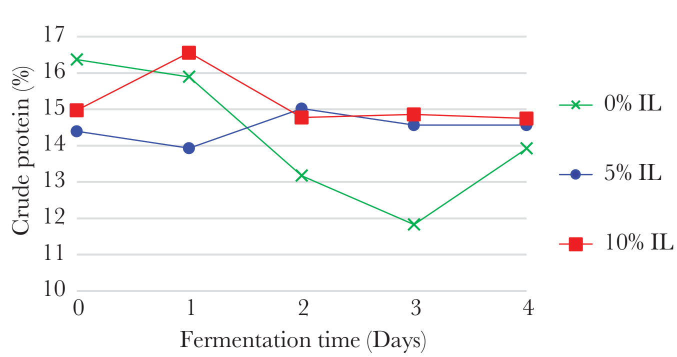

Figure 3. Effect of yeast inoculum (YI) levels and fermentation days on the crude protein content (%) of the fermented food.

this study was 8%: a good amount, based on the protein value of cassava (2.4%). However, these values are lower than those reported by Aruna *et al.* (2017), who enriched yam (*Dioscorea* spp.) peelings using a *Saccharomyces cerevisiae* medium and found a significant increase in TP (4.38 to 13.37%) after a 96 h fermentation.

No differences resulting from the inoculum levels or fermentation times were found in IDDM (Table 2). In average, IDDM was of 80%; these data are slightly higher than those reported by Flores *et al.* (2005), who used *Penicillum roqueforti*- and *Lactobacillus plantarum*-based cultures on coconut paste and found an IDDM of 72 to 75%.

Microbiological analyses

We found a significant interaction of the studied factors regarding yeast growth. No difference between the inoculum levels and fermentation days was found on days 0, 1, 2, and 4; however, on day 3, the addition of 5% YI increased yeast growth (Figure 4).

Table 2. Effect of yeast inoculum levels and fermentation times on the chemical and microbial parameters of the fermented foods.

Factors	Dry matter (%)	True protein (%)	[1]DIMS (%)	[2]BacLac (Log_{10} UFC mL^{-1})
Yeast inoculum levels (%)				
0	34.01a	7.80a	79.64a	6.35a
5	34.32a	7.82a	79.67a	6.36a
10	32.71a	8.28a	81.14a	6.38a
EE±	0.4349	0.2147	0.5828	0.061
Fermentation time (Days)				
0	33.07a	8.19a	79.57a	6.19b
1	33.85a	8.20a	80.10a	6.44ab
2	33.78a	7.85a	81.01a	6.67a
3	33.47a	7.40a	79.46a	6.30b
4	34.22a	8.20a	80.62a	6.21b
EE±	0.5615	0.2772	0.7524	0.061

Means with different letters in the same column are statistically different (Tukey, $p<0.05$).
[1]IDDM (DIMS)=*In situ* degradation of dry matter; [2]LacBac (BacLac)=Lactic bacteria.

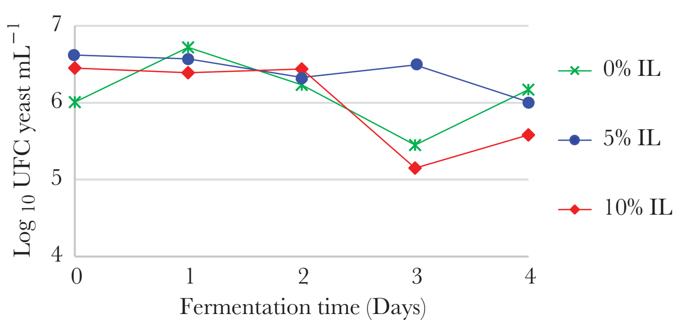

Figure 4. Effect of yeast inoculum levels and fermentation times on the fermented cassava's yeast growth (Log_{10} UFC mL^{-1}).

Yeasts with 0% YI were found on day 0, probably due to the yeasts that are naturally present in sugarcane (Valiño *et al.*, 1994a; 1994b) and to their increase when they are cut 24 h before grinding, mixing, and fermenting the cassava-based feed (Elías *et al.*, 1990).

On the one hand, the lack of growth response in yeast to the addition of YI on days 0, 1, and 2 could be caused by the alkaline pH (Figure 1), which did not allow an optimal yeast growth. On the other hand, the growth observed on day 3 of fermentation after a 5% YI had been added (Figure 4) matches the lower pH found in this treatment (Figure 1).

We previously mentioned that yeast growth is associated with an acid pH. Díaz-Plascencia *et al.* (2011) studied four different substrates with *Saccharomyces cerevisiae* yeast inoculum under submerged solid fermentation conditions and found that yeast concentrations increased with a pH of 3.05 to 5.86. Fonseca-López *et al.* (2018) produced feed for bovines based on SSF-fermented carrot. They observed that a ≥6.05 pH has a negative impact on fungi and yeast growth, as was the case in this study.

No effect was found on lactic bacteria due to the studied inoculum levels. However, there were differences due to fermentation times: the highest growth was found on day 2 (Table 2).

Lactic bacteria growth is associated with a low pH. Fonseca-López *et al.* (2018) found that lactic bacteria did not grow in carrot-based fermented foods with a pH of 6.05; however, they did grow adequately with a pH of 5.

The high pH values found in this study were probably the factor that most affected yeast and lactic bacteria growth. Meanwhile, the CP, TP, and IDDM values found in the cassava-based fermented food support their suitability as part of the bovine diet. We therefore recommend conducting behavior tests.

CONCLUSIONS

The yeast inoculum and fermentation days did not increase true protein contents in the cassava-based fermented food. The data analysis suggests that —given its crude and true protein contents and *in situ* degradation of dry matter— the cassava-based fermented food, with no yeast inoculum and a 24 h fermentation, can be used as an energy-protein supplement in bovine diet that improves production and, possibly, contributes to a reduction of production costs.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Coffee (*Coffea arabica* L.) harvesting time and its influence on the seed quality of the Costa Rica 95 and Garnica varieties

Del Porte-Morales, Cielo G.[1]**; García-De Los Santos, Gabino**[1*]**; Aguilar-Rincón, Víctor H.**[1]**; Hernández-Livera, Adrián**[1]**; Escamilla-Prado Esteban**[2]

[1] Colegio de Postgraduados, Postgrado en Recursos Genéticos y Productividad-Programa de Producción de Semillas del Colegio de Postgraduados, Campus Montecillo, carretera México-Texcoco km 36.5, Montecillo, Estado de México, México, C. P. 56230.

[2] Universidad Autónoma Chapingo, Centro Regional Universitario Oriente, Carretera Huatusco-Xalapa km 6, Huatusco, Veracruz, México, C. P. 94100.

* Correspondence: garciag@colpos.mx

ABSTRACT

Objective: To determine the impact of the harvesting time of two coffee varieties on the physical quality and viability of seeds, using the tetrazolium testing.

Design/Methodology/Approach: The research was carried out using a completely randomized design; the evaluation of the embryo viability was based on a five-color pattern staining design. The Garnica and Costa Rica 95 varieties and two harvesting times were used (December and January). An analysis of variance and a Tukey's means comparison test ($p \leq 0.05$) were carried out, using the SAS 902 software.

Results: The study varieties showed significative differences in all the variables related to the physical quality of the seeds, including volumetric weight and weight of 1,000 seeds. The best seed viability was obtained during the harvest carried out in January.

Study Limitations/Implications: The results obtained are limited to the varieties in question, as well as the environmental conditions and period during which the said varieties were evaluated.

Conclusions: The harvesting time of the two varieties of coffee has an influence on the physical characteristics of the seeds and on the viability, evaluation carried out using tetrazolium.

Keywords: viability, tetrazolium, coffee, physical characteristics.

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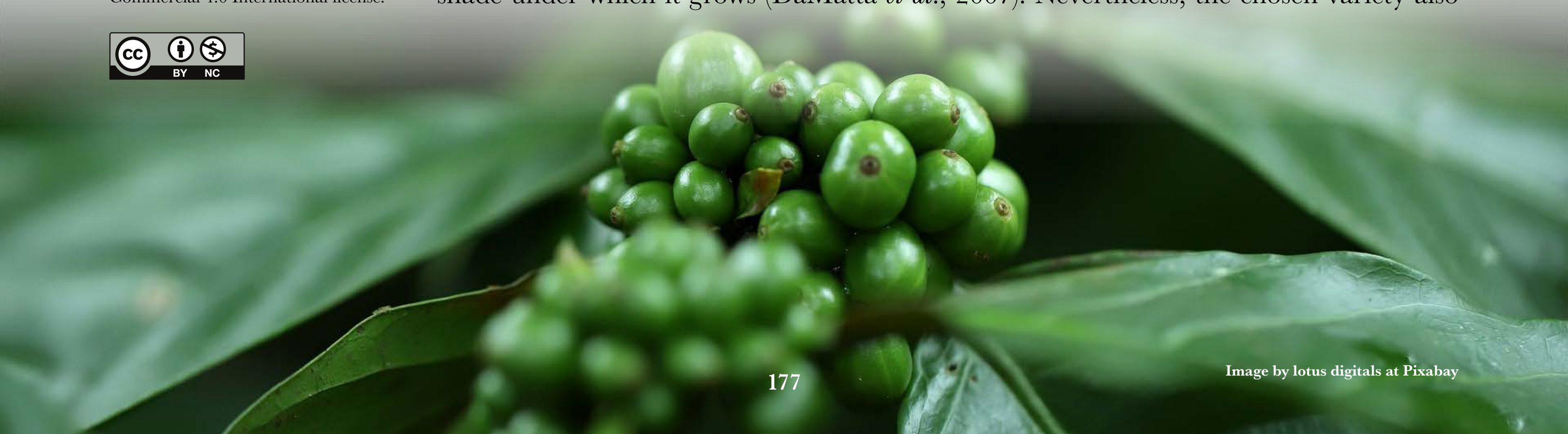
INTRODUCTION

Genetically speaking, most coffee (*Coffea arabica* L.) varieties worldwide are very similar; however, morphologically speaking, they are very different, and their fruits have contrasting pre- and post-harvest qualities (Steiger *et al.*, 2002).

In the international market, some environmental factors are known to influence the quality of coffee, such as the altitude of the coffee plantation (Bertrand *et al.*, 2006) and the shade under which it grows (DaMatta *et al.*, 2007). Nevertheless, the chosen variety also



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plays a key role in any production system, because the quantity and quality of the harvested fruits depends on the genotype selection and its capacity to adapt to the environment (Hein and Gatzweiler, 2005; Kathurima *et al.*, 2009).

Good quality seeds are required to produce excellent plants; consequently, several studies have been carried out to establish the optimal harvesting time that results in good quality seeds. The main harvesting time of coffee in Mexico goes from January to March (ASERCA, 2010); however, in some regions, the harvesting time starts in December. Nevertheless, this harvesting time is very long and the ripening of the fruits varies widely. Consequently, the seeds produced have a highly variable physiological quality.

In order to establish coffee plant greenhouses, the best seeds from well-ripened fruits must be chosen. These fruits must be harvested from healthy and well-developed plants, whose productivity has been proved. They must be 4-7 years old and free of pathogens and diseases. Usually, the best fruits can be in the middle part of the branches, in the center of the plant (Aranda-Bezaury *et al.*, 2017). However, the fruits are usually harvested regardless of their color or ripening stage. They are taken from different parts of the plant and, sometimes, they are harvested at different times without a post-harvest classification process. Usually, this situation has an impact on the different physiological or physical quality of the coffee seeds which, on their turn, produce seedlings of different germination and vigor. Therefore, the objective of this research was to determine the importance of the harvesting time of two varieties of coffee and its relationship with the physical quality and viability of the seed, which was evaluated using the tetrazolium testing.

MATERIALS AND METHODS

Plant material and experiment location

Seeds from the Garnica and Costa Rica 95 varieties were used. They were harvested in Zacamitla, Ixhuatlán del Café, Veracruz, Mexico. The laboratory stage was carried out in the Laboratorio de Análisis de Semillas of the Postgrado en Recursos Genéticos y Productividad-Programa de Producción de Semillas, Colegio de Postgraduados - Campus Montecillo.

Harvest

Two harvests were carried out: one in mid-December and the other in mid-January. The fruits were harvested by hand when they reached an optimal ripening —*i.e.*, when the color of the fruits ranged from "Cherry" red to a dark red (Aparecida-Sagio *et al.*, 2013). Immediately, the pulping was carried out and the resulting raw material was left to ferment for 24h to obtain the seed. Subsequently, the seed was washed and dried in the shade. Once it was dry, the physical quality and viability were determined using the tetrazolium testing.

Treatments and experimental design

A 2×2 factorial experiment was conducted, using the following four treatments which were the result of combining two varieties and two harvesting times: Treatment 1: Costa Rica variety, harvested in December (CR-D); Treatment 2: Costa Rica variety, harvested in

January (CR-J); Treatment 3: Garnica variety, harvested in December (GAR-D Treatment 4: Garnica variety, harvested in January (GAR-J).

A completely random design was used, and the experimental units were made up of Sanitas towels with 100 seeds. Each treatment had four repetitions.

When the seed had reached the appropriate humidity, the physical quality variables were determined. Subsequently, the embryo viability was established. All the quality evaluations of the seeds were carried out according to the international standards of the ISTA (ISTA, 2015).

Seed quality characteristics

Humidity Content (HC). In order to determine the humidity content, the stove dried method was used (Central Scientific Division of CENCO). The seeds were dried at 103 °C for 20 h. Two repetitions were carried out to determine humidity content, using 5-cm diameter aluminum boxes with tops. Subsequently, 10 g of pure coffee seeds were placed in the boxes. Once the box and the seeds had been weighted, the boxes were placed over the tops and put in the stove. After the dry procedure, the boxes were taken out of the stove and they were immediately weighted. A $\leq 0.2\%$ difference was recorded and therefore humidity determination was considered appropriate (ISTA, 2009; MAPA, 2009).

The humidity content was calculated using the following formula:

$$\%Humidity = M_2 - M_3 \times \frac{100}{M_2 - M_1}$$

Volumetric Weight (VW). The volumetric weight was determined from a 100 g pure coffee seed sample, which was poured into a 250-mL test tube. Subsequently, the value was determined based on the volume it occupied. The data were obtained from each of the four repetitions per treatment. The volumetric weight was calculated as follows:

$$VW = \left(\frac{100g\ seed\ weight}{Volume\ occupied\ by\ the\ 100g\,(ml)}\right) \times 100$$

Weight of 1,000 seeds (WTS). The weight of 1,000 seeds was determined counting and weighting eight repetitions of 100 coffee seeds. Based on the data obtained, the mean, variance, standard deviation, and variation coefficient were calculated. When the coefficient of variation was $<4\%$, we considered that the data was correct. The weight of a thousand seeds was obtained multiplying tenfold the arithmetic mean of the eight repetitions; the result was expressed in grams (ISTA, 2015).

Shape of the seed. The different shapes of the seeds that prevail in coffee varieties are part of their physical quality. They are frequently considered as characteristics that are sometimes correlated with the germination behaviour, viability, and vigor of the seeds. Based on their shape, coffee seeds are classified as: flat coffee seed, peaberry coffee seed, triangular coffee seed, and black Ivory coffee seed. Therefore, a physical characterization of the seeds of all treatments —resulting from the combination of the two study varieties and the two harvesting dates— was carried out. Subsequently, the percentage of composition was determined based on the different shapes of the coffee seeds.

Digital analysis of the coffee seed images. As part of the characterization of the physical quality, coffee seed images of the four evaluated treatments were processed. The said images were taken with an Epson scanner and were processed in a HP laptop, using the version 1.46r of the ImageJ software (Ferreira and Rasband, 2012). Four-hundred coffee seeds —divided up among four repetitions of 100 seeds— were used per variety. The following variables were recorded: area, perimeter, length, and width of the coffee seeds (Linskens and Jackson, 1992).

TZ viability (TV). In order to extract the embryos, a 100-coffee seeds sample was taken from each of the treatments —obtained from the combination of the varieties and the harvesting times— following the Dias and Silva (1998) methodology, with some adaptations (Figure 1).

The parchment of the coffee seed was extracted from 100 seeds. Subsequently, they were placed into distilled water for 24 h, at room temperature. Afterwards, lengthwise sections and cross sections were made to allow the water to enter and to soften the seeds.

After the sections were made, the water of the seeds was changed, and they were submerged again in distilled water for 24 h to perform the extraction. Later, the embryos were extracted and placed in distilled water. Figure 2 shows the modified extraction sequence.

A 1.0%-concentration tetrazolium solution (TZ, 2,3,5-tryphenil tetrazolium chloride) was prepared, diluting one gram of tetrazolium salt in 100 mL of distilled water, with a 7.0 pH.

The embryos were placed in the solution and then moved to a light-less chamber for 24h. Subsequently, the TZ excess was eliminated, and the stained embryos were observed. Their coloring varied from pink to red, indicating that the embryos were alive. Meanwhile, unstained embryos were considered dead, because they did not show any reaction (França-Neto *et al*., 1998).

Evaluation of the embryos. The embryos were placed in a previously soaked Sanita paper towel, to avoid dehydration. Subsequently, they were put under an Olympus SZX7 stereoscopic microscope using tweezers and the staining pattern was determined. The number of viable embryos was determined according to the staining pattern obtained, using the Munsell color chart (Munsell Color Charts, 1977) and they were classified into several categories: 2.5 R 4/10 dark red (viable embryo); 2.5 R 7/8 soft red (viable embryo);

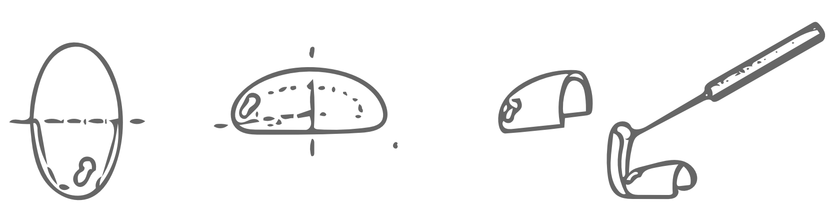

Figure 1. Extraction of a coffee seed embryo (Días and Silva, 1998).

Figure 2. Embryo extraction (adapted methodology). a) First 24h of imbibition. b) Embryo localization. c) Separation of the endosperm. d) Endosperm sheet with an embryo. e) Sheet with an embryo placed in water for 24h. f) Embryo without endosperm. The viability percentage was calculated using the following formula:

$$\%\ viability = \frac{mean\ value\ of\ viable\ embryos}{Number\ of\ coffee\ beans\ used\ per\ repetition}.$$

2.5 R 7/4 dark pink (viable embryo); 1.5 R 8/4 soft pink (viable embryo); and 2.5 R 8/2 white (non-viable embryo) (Figure 3).

Statistical analysis

The normality assumption, the homogeneity of variances, and the multi-collinearity were verified before the statistical analysis was carried out. The non-normal data were transformed using the $\sqrt{X/100}$ arcsine function. An analysis of variance and a Tukey's

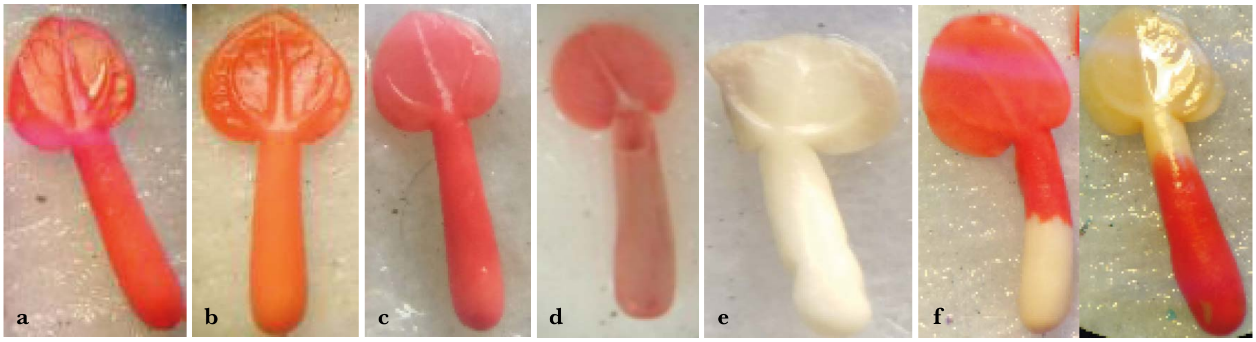

Figure 3. Staining pattern of coffee embryos. a) 2.5 R 4/10 dark red (viable embryo), b) 2.5 R 7/8 soft red (viable embryo), c) 2.5 R 7/4 dark pink (viable embryo), d) 1.5 R 8/4 soft pink (viable embryo), e) 2.5 R 8/2 white (non-viable embryo), and f) Some essential parts of an embryo without staining (non-viable embryos).

mean comparison test (p≤0.05) were carried out for each of the response variables, using the SAS statistical package software (SAS Institute, 2002).

RESULTS AND DISCUSSION

Table 1 shows the behavior of the quality variables of the coffee seeds.

There were significant differences (p≤0.001) in the physical quality variables, volumetric weight, and weight of 1,000 seeds of coffee. There were significative differences, both in the independent effects and the interaction (V×E) variables. The differences found in these physical quality variables of coffee seeds indicate a good agronomic management in the field. The environmental conditions during the crop development have a high impact on management. Ultimately, these environmental conditions have an impact on the seeds, although the genetic component should not be forgotten. Additionally, the significative effect during the harvesting times indicates that the quality of the seeds is determined in part by the moment in which the harvest is carried out.

In crops such as maize, the genetic component has greater influence in attributes such as volumetric weight and weight of a thousand seeds than the environment itself (Flint-García *et al.*, 2009; Torres *et al.*, 2010).

Table 1 also shows the results of the viability evaluated using the TZ testing, according to the harvesting time of both varieties. There were significative differences in the variables (p≤0.01) and in the harvesting times (p≤0.001), while the interaction was not significative. The germination potential of a seed is frequently evaluated using the TZ testing which has a high correlation with the behavior of the seeds, when they are subject to a direct evaluation in a standard germination evaluation. In the case of the variables evaluated in this work, Costa Rica 95 had a different viability behavior. Perhaps the genetic factor is involved in this behavior. This situation can be related to the non-significative results of the interaction, which indicates that, in both varieties, TZ-evaluated viability can also be determined by the genetic fact and the harvesting time. However, the resulting significance indicates that harvesting during different periods has an influence on the differential quality of coffee seeds. If we relate the obtained viability results with the harvesting time, it is not surprise that the coffee fruits selected for the production of high-quality coffee seeds are chosen from intermediate and late harvests and rarely from early harvests. These fruits are taken from the middle of the plant and from the middle of the branches: the areas where fruits of even color and size can be located. This practice is always reflected in better quality coffee seeds (Aparecida-Sagio

Table 1. Mean squares of the quality variables of coffee seeds from two varieties of coffee in two harvesting times.

Variable	Sources of Variation			Coefficient of variation	R^2
	Varieties (V)	Harvest time (H)	V×H		
Volumetric weight	16.81***	23.04***	27.04***	0.82	0.97
weight of 1000 seeds	27.73***	41.84***	44.16***	5.84	0.63
Viability with TZ (%)	64.00**	196***	ns	2.87	0.75

, * Significative with α=0.01 and 0.001, respectively; ns=not significative.

et al., 2013). Both the coefficients of variation and the coefficients of determination were acceptable, which shows the goodness of fit of the model regarding the importance of the variables considered by the said model.

Means comparison

Table 2 shows the average behavior of the varieties (Tukey, 0.05) regarding the physical quality and viability evaluated with TZ testing, according to the harvesting times. Regarding the effect of the harvesting time, the best behavior for all the variables took place when the seeds were harvested in January, although Garnica had the best behavior.

The Garnica variety that had the highest number of stained embryos (96.5%); this percentage belonged to the January harvest. This could be the result of the smaller size of the seed of the Costa Rica 95 variety (Aguilar-Vega, 1995), which suffers less damage and consequently has an enhanced viability. In all the cases, when comparing the physical characteristics of the coffee seeds —for example, those obtained subjecting the seeds to image digitalization, such as area, perimeter, length, and width (data not shown)—, differences were found between the two evaluated varieties. In all cases, the best quality of coffee seeds —according to the abovementioned physical parameters— was obtained by Garnica. This matches the results obtained.

Meanwhile, in this study, the physical characterization data was recorded according to the prevailing shape or type of coffee seeds in both coffee varieties (data not shown). Samples were gathered both from the January and December harvests. The quantity of the flat coffee seed —which is the biggest and more even-shaped seed and the one that prevailed— was quantified as the number of coffee seeds/kilogram. As a result, Garnica (679.9) obtained less flat coffee seeds than Costa Rica 95 (908.8). However, these data were remarkably similar in both harvesting times, matching the results of Castillo-Zapata and Moreno-Ruiz (1988), regarding the shape and types of coffee seeds (peaberry. "monster," and triangular coffee seeds, etc.), which are determined by genetic factors and meiotic irregularities rather than environmental or management factors. Further research is required on this matter. This research must include a detailed follow-up of field data and a combination of study factors —such as position of the fruits in the plant and the branches,

Table 2. Quality variables of coffee seeds from two coffee varieties in two harvesting times.

Source of variation	Variable		
Harvest time	**Weight of 1000 seeds**	**Volumetric weight ($g\ mL^{-1}$)**	**Viability with TZ (%)**
January	27.46 a	51.83 a	98.00 a
December	25.17 b	49.43 b	91.00 b
Varieties			
Garnica	27.24 a	51.65 a	96.50 a
Costa Rica 95	25.38 b	49.60 b	92.50 b
MSD	1.12	0.45	2.95

MSD=minimal significant difference. Mean with the same letter inside the columns are not significatively different (Tukey, α=0.05).

ripening level of the fruit, harvesting time, size and shape of the coffee seed, etc.—, in order to determine their influence on the quality of the coffee seed.

CONCLUSIONS

January was the best harvesting time, which occurred simultaneously with a better physical quality of the coffee seeds, recording the highest volumetric weight, the weight of 1,000 coffee seeds, and viability. Garnica was the variety that behaved the best in all the variables (both physical quality and viability of the coffee seed evaluated with TZ testing). The viability percentage was higher in the coffee seeds from both varieties harvested in January.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Reallocation of water in agriculture under drought conditions as economic efficiency maximizer

Botello-Aguillón, César[1*]; Valdivia-Alcalá, Ramón[1]; Hernández-Ortiz, Juan[1]; Sangermán-Jarquín, Dora M.[2]; Gutiérrez-García, Francisco G.[1]

[1] Universidad Autónoma Chapingo, Carretera México-Texcoco km 38.5, Chapingo, Estado de México, México, C. P. 56230.

[2] Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, Carretera Texcoco-Los Reyes km 13.5, Coatlinchán, Estado de México, México, C. P. 56250.

* Correspondence: cbotelloa@gmail.com

ABSTRACT

Objective: To analyze the water market scheme for water rights transfer, which could enable the increase of the economic efficiency of water use in the Irrigation District 011 - Alto Río Lerma.

Design/Methodology/Approach: Using linear programming, a first model was developed to determine shadow prices in three water scarcity scenarios (15, 30, and 50% water resources reduction) and to compare them with the irrigation fees currently paid in Irrigation District 011. The second model established a water market scheme, using the same water scarcity scenarios (15, 30, and 50%). This model was developed to compare the net profit of the producers within and outside the water right transfer market.

Results: The average shadow price of water is MN$ 3.9 m^{-3}; this amount is higher than the irrigation fee currently paid (MN$ 0.15 m^{-3}). The water transfer percentages are 25.8, 29.1, and 36.1%, obtaining 7.6, 7.4 and 11.7% net profit, respectively, for each water scarcity scenario (15, 30, and 50%).

Study Limitations/Implications: The research was carried out based on the data from two out of the 11 irrigation modules included in Irrigation District 011. These modules are the most representative, both in extension and crop variety.

Findings/Conclusions: The existence of a water market confirms the advantages of an increase in the net profit of the producers under drought conditions, included within the area of Irrigation District 011.

Keywords: Water scarcity, water market, optimization, productivity.

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INTRODUCTION

For a long time, when water seemed to be abundant, people behaved as if this resource had no value. The fourth principle of the International Conference on Water and the Environment established that "water has an economic value in all its competing uses and should be recognized as an economic good" (ONU, 1992). Theoretical development has proved that water has a series of social, economic, and cultural characteristics. Therefore, water is a special resource; however —like other production resources—, it has an economic value (Hanemann, 2006).

Image by S. Hermann & F. Richter at Pixabay

There are many methods to measure the value of water and its contribution to the different productive processes. Giving an appropriate value to water can become a useful tool to reallocate it to more efficient uses (Young and Loomis, 2014).

In agriculture, increasing the economic efficiency through the reallocation of water to crops with higher value is a way to guarantee that the users can increase their profits. In a mathematical programming model, the economic criteria behind the water reallocation to crops complies with the equivalence between the marginal revenue and the marginal cost (Hazell and Norton, 1986; Beattie and Taylor, 1985). In those cases where the government is involved in the decision-making process regarding the use of water, shadow prices must be estimated, in order to guide the efficient allocation of water (Young and Loomis, 2014).

In its normative and positive forms, mathematical programming shows the importance of its use in the decision-making process about water allocation in agriculture (Filippi *et al.*, 2017; Ren *et al.*, 2017; Ahmad *et al.*, 2018; Li *et al.*, 2018; Zhang and Guo, 2018).

The application of mathematical programming in agriculture allows to reallocate farm and regional resources, taking into account a wide range of situations, such as price variations and availability, new and more profitable production activities, and market or institutional limitations, among others (Hazell and Norton, 1986).

In Irrigation District 011 (ID 011), the agricultural production yield has been impacted by the reduction of the volume in the whole basin. Several studies that tackle the valuation of water within ID 011 have proposed some methodologies to estimate the value of water, mainly in water scarcity scenarios (Florencio-Cruz *et al.*, 2002; Rubiños-Panta *et al.*, 2007; Rodríguez-Flores *et al.*, 2019; Pineda-Espejel, 2019). Overall, these researches focus on the analysis of water optimization aspects between the different crops and the estimation of marginal productivity. Rubiños-Panta *et al.* (2007) and Rodríguez-Flores *et al.* (2019) have analyzed the water right transfer and the water reallocation between the irrigation modules of ID 011.

The existence of water transfer is well known, not only within agricultural activities, but also towards the industry and the services in the area of interest (Sosa-Márquez *et al.*, 2019). Additionally, the agricultural sector is the main water consumer in Mexico and worldwide (FAO-WWC, 2015) and, therefore, looking for short-term alternatives —such as the opportunity to develop a water market within the ID 011— remains an important task. Consequently, the aim of this study was to understand water productivity in two irrigation modules of the ID 011, using them as reference to compare the irrigation fees and to analyze the water market scheme for water right transfer between the producers of the said irrigation modules, which can enable an increase in the economic efficiency of water use.

MATERIALS AND METHODS

The research was carried out in the Irrigation District 011 - Alto Río Lerma, in southern Guanajuato, Mexico. It is located between 19° 55’ and 21° 52’ N and 99° 39’ and 102° 05’ W, at an altitude of 1,722 m. It is part of the Lerma-Chapala drainage basin, where 30% of the industrial production and 12.5% of the agricultural production of the country take place; in addition, 75% of the water of this area is used for agriculture and livestock raising (Fernández-Durán and Lloret, 2016).

Data were gathered from the M02: Salvatierra and M05: Cortázar agricultural production modules. These modules share the same source for the gravity-fed irrigation model and have a higher water demand (29% of the available total); they include representative regional crops. The data used for this study were: yield, average rural price, production costs, sown area, and water volume used. This information was provided by the Head of the Irrigation District 011 - Alto Río Lerma and the Limited Liability Company of the ID 011.

Using this information, a lineal programming model was developed. This model was used to establish both the maximum profit based on the availability of the fixed resources of the farm and crop definition that sets the pattern of the crops that have been sown in ID 011. We used a base model with the real crop pattern for the 2016-2017 agricultural period and the total water volume available that year.

The 2016-2017 information was used because that was a typical year: the water volume remained constant in the dams that supply the ID 011, reaching a historical level. Faced with water scarcity, producers only sow during one cycle (autumn-winter/spring-summer). Several scenarios were developed using different water scarcity levels (15, 30, and 50% water reduction) to carry out agricultural activities.

The mathematical representation of the model can be expressed as follows (Kaiser and Messer, 2011).

Objective function:

Maximize $$Z=\sum_{j=1}^{n} c_j x_j \quad (1)$$

Subject to:

$$\sum_{j=1}^{n} a_{ij} x_j \leq b_i \text{, for all } i=1,\ldots, m \quad (2)$$

$$x_j \leq \beta x_j^{year\ base} \quad (3)$$

$$\text{and } x_j \geq 0 \text{, for all } j=1,\ldots, n \quad (4)$$

Where: x_j=is the j-i^{th} activity of the producer, the number of hectares to be sown with a given crop; c_j=is the net profit margin forecast (also known as net prices) of the j-i^{th} unit of the activity (pesos per hectare); a_{ij}=quantity of the i-i^{th} resource (water, land) required to produce a unit of the j-i^{th} activity; m is the number of resources, therefore $i=1, \ldots, m$; b_i=is the amount of the i-i^{th} available resource (water, land); β=allowable percentage of the j-i^{th} activity of the producer, dimensionless.

For the development of the empirical model, the crops established in the M02: Salvatierra and M05: Cortázar modules were used —including barley, maize, sorghum, and wheat. They represent 83.1% of the cultivated area and 77.3% of the water delivered to the modules (Table 1).

The net prices were calculated as the difference between the gross income (yield multiplied by the average rural price) and the production cost per sown hectare —not including water costs, which are precisely the value that we are looking for.

Table 1. Crops established in the M02: Salvatierra and M05: Cortázar modules in the 2016-2017 period.

Crop	Area (ha)	Volume of water (dam^3)	Crop	Area (ha)	Volume of water (dam^3)
Garlic (*Allium sativum*)	46.4	275.6	Strawberry (*Fragaria* × *ananassa*)	78.7	508.2
Alfalfa (*Medicago sativa*)	1378.1	17421.2	Beans (*Phaseolus vulgaris*)	1179.5	14996.7
Fodder oats (*Avena sativa*)	81.8	760.0	Bean (*Phaseolus vulgaris*)	30.4	138.9
Broccoli (*Brassica oleracea* var. *italica*)	743.2	5653.7	Chickpea (*Cicer arietinum*)	378.2	1778.6
Peanut (*Arachis hypogaea*)	143.6	721.6	Guava (*Psidium guajava*)	7.5	41.6
Zucchini (*Cucurbita* pepo)	50.9	457.8	Tomato (*Solanum lycopersicum*)	10.1	40.5
Sweet potato (*Ipomoea batatas*)	87.9	833.6	Cabbage (*Lactuca sativa*)	927.1	5546.1
Barley (*Hordeum vulgare*)	8466.9	65900.3	Corn (*Zea mays*)	178.9	2265.5
White onion (*Allium cepa*)	235.7	2868.1	Grain corn (*Zea mays*)	19331.9	102696.6
Chayote squash (*Sechium edule*)	2.8	30.3	Sorghum (*Sorghum bicolor*)	5887.5	21014.7
Coriander (*Coriandrum sativum*)	2.1	51.8	Green tomatoes (*Physalis philadelphica*)	818.7	10242.6
Cauliflower (*Brassica oleracea* var. *botrytis*)	154.7	1113.8	Wheat (*Triticum durum*)	6903.3	71033.4
Asparagus (*Asparagus officinalis*)	484.5	2592.7	Carrot (*Daucus carota*)	734.4	7951.3

Source: Table developed by the authors using information of the Office of the Head of ID 011 and the Limited Liability Company of the ID 011.

Water volume and available area vary according to the agricultural cycle and the irrigation method. Water and land restrictions were considered. The former amounted to 337,007 dam^3 (*i.e.*, the water volume used by M02: Salvatierra and M05: Cortázar modules during the 2016-2017 period). The latter matches the sowing area of each module (Table 2).

Subsequently, a second model was developed. This model enabled —within the same water scarcity scenarios (15, 30, and 50% water reduction)— the existence of a water market scheme between the producers of the two irrigation modules. This model was based on chapter V of the Ley de Aguas Nacionales (DOF, 2020), which allows the transfer of

Table 2. Resources restriction for the lineal programming model.

Type of irrigation	Cycle	M02: Salvatierra		M05: Cortázar	
	Resource	Land (ha)	Water (dam^3)	Land (ha)	Water (dam^3)
Surface	Fall-Winter	12092.3	50955.1	11930.7	73949.4
	Spring-Summer		46282.9		5979.9
	Perennials		10927.6		161.8
	Second crops		18045.8		32159.6
Pumping	Fall-Winter	4075.9	22418.8	7184.0	34658.9
	Spring-Summer		11840.9		346.3
	Perennials		4894.5		4609.9
	Second crops		5915.5		13859.9
	TOTAL	16168.2	171281.3	19114.7	165725.8

Source: Table developed by the authors using information of the Office of the Head of ID 011 and the Limited Liability Company of the ID 011.

concession arrangements for the exploitation or use of national waters between economic agents and sectors.

The lineal programming models were processed using the LINDO 6.1 (Lineal Interactive Discrete Optimization) software. The results were processed with a three-part analysis: the first part covers the shadow prices; the second includes the water volume allocated to the modules; and the third shows the net income of the irrigation modules.

RESULTS AND DISCUSSION

Using the resource optimization model supported by the availability water restriction, the shadow prices of gravity-fed and land were initially obtained (Table 3).

When shadow prices reach a zero value, it does not mean that the resource has ran out completely and, therefore, it is free of charge. This was the overall case of the shadow prices of the land resource. The results showed that the water shadow price of the base model was MN\$3.9 m^{-3}; this price is much higher than the price paid by the users (MN\$0.15 m^{-3}). A similar relation was found by Martínez-Luna *et al.* (2021), who recorded a MN\$1.44 m^{-3} shadow price for the Irrigation District 100, in Alfajayucan, Hidalgo, Mexico. That price is higher than the irrigation fees paid by the producers (MN\$0.02 m^{-3}). Ramírez-Barraza *et al.* (2019) carried out a study in the Comarca Lagunera and recorded a MN\$0.91 shadow price; this price is higher than the price paid by the producers of the study area.

The existence of a water market in ID 011 allows the reallocation of water volume to other modules. Figure 1 shows the water transfer between agricultural cycles in M02 and M05 modules. Additionally, it enables a comparison with the established volumes, when the district lacks a water market.

In view of a potential water market, water was used in the agricultural cycles in which the highest shadow prices are obtained. This behavior shows that —within the irrigation modules of a district—the resource was bought and sold in those places where high profit crops are sown, or water demand is lower. Rodríguez-Flores *et al.* (2019) recorded a different situation in their study. They evaluated a formal water market for all the ID 011, where the four modules with highest overall shadow prices imported the resource.

Table 3. Shadow prices of gravity-fed and land per irrigation module (MN\$ dam^{-3}).

Availability	**100%**		**85%**		**70%**		**50%**	
Resource	**M02**	**M05**	**M02**	**M05**	**M02**	**M05**	**M02**	**M05**
Water								
F-W	1284	1371	1284	1371	3484	1371	4246	1371
S-S	2084	3872	2084	3872	2084	3872	2631	3872
Perennial	1964	12771	1964	12771	1964	12771	1964	12771
Second crops	4222	3640	4222	3640	4222	3640	4222	3640
Land								
June	0	9987	0	0	0	0	0	0
July	0	9953	0	17027	0	12687	0	3507

Source: Table developed by the authors using information of the outputs of the MPL of the LINDO 6.1.

The main aim of this water market projection is to prove the economic effect that it can have on the ID 011 users. In addition, along with an optimal crop pattern, it can increase their net profit. Table 4 shows the results of the M02 and M05 modules net income. A water market can indeed favor the total net income in all scarcity scenarios.

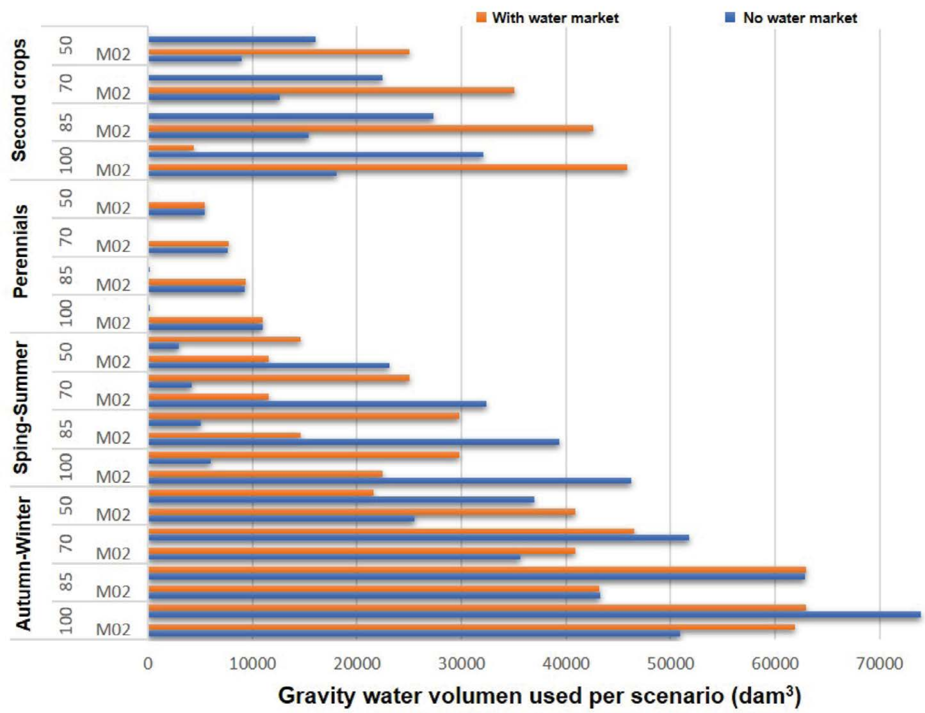

Figure 1. Water volume for gravity-fed irrigation used per module and each availability scenario. Source: Table developed by the authors using information of the outputs of the MPL of the LINDO 6.1.

Table 4. Net income of the M02 and M05 irrigation modules in different availability and water market scenarios (millions MN$).

Water availability	Net income in millions of Mexican pesos					
	No water markets			With water markets		
	85%	70%	50%	85%	70%	50%
M02: SALVATIERRA						
Spring-Summer	187.31	171.28	129.05	187.18	184.19	184.19
Fall-Winter	195.91	181.44	159.6	137.07	126.73	126.73
Perennials	17.95	14.73	10.43	18.08	14.81	10.46
Second crops	64.76	53.33	38.09	180.04	148.25	105.86
M05: CORTÁZAR						
Spring-Summer	88.57	73.36	53.09	88.7	66.21	31.97
Fall-Winter	29.14	25.67	21.04	124.99	106.46	66
Perennials	1.76	1.45	1.03	0.88	0.88	0.88
Second crops	99.87	82.32	58.91	0.49	0.49	0.49
Total:	685.27	603.58	471.24	737.43	648.02	526.58

Source: Table developed by the authors using information of the MPL of the LINDO 6.1.

CONCLUSIONS

The irrigation fees paid within ID 011 do not represent the actual value of water. An adjustment in the fee prices could benefit ID 011. Such benefits would include better management or improvements to the current hydro-agricultural infrastructure that would enable a higher irrigation efficiency for the distribution of water to the local users.

Further crop pattern analysis must be carried out in ID 011. Additionally, crops with better characteristics (*i.e*., profitable, low investment, existing market, etc.) must be taken into account as an option for future agricultural years. Crops such as alfalfa, oats, maize, and sorghum should not be sown because of their low profitability and/or water demand. Water commercialization is an efficient mechanism in the economy of the hydric resources. It should spark an interest in water governance for lost markets. This situation could be the result of legal restrictions related to the commercialization of water rights. It can improve the efficient use of water in agriculture, supplying the forecasted water demands, resulting from population growth. The results of this research lay the foundations for the generation of a market policy for right water transfer in ID 011. This policy should motivate both buyers and sellers to evaluate water use strategies related to scarcity water values. In addition, we must be aware of the costs of the infrastructure involved in the water market development.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Seed yield variables of five wild Poaceae species in La Siberia, Chapingo, México

Rosas-Ramos Xuxan Alyn[1]; **Castrellón-Montelongo Jorge Luis**[2]; **Velázquez-Martínez Mauricio**[3]; **Hernández-Martínez Rosendo**[4]; **Hernández-Guzmán Filogonio Jesús**[5*]; **Zaragoza-Ramírez José Luis**[2]

[1] Comisión de Recursos Naturales y Desarrollo Rural. Av. Año de Juárez 9700. Col. Quirino Mendoza, Pueblo de San Luis Tlaxialtemalco, Xochimilco. C.P. 16610. Ciudad de México, México.
[2] Universidad Autónoma Chapingo-Departamento de Zootecnia. Km. 38.5 Carretera México-Texcoco Chapingo, CP. 56230. Texcoco, Estado de México, México. .
[3] Universidad Politécnica de Francisco I. Madero. Domicilio conocido s/n, CP. 42660. Tepatepec, Francisco I. Madero, Hidalgo, México.
[4] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). Carretera San Luis Potosí-Matehuala km 14.5, Ejido Palma de la Cruz, Soledad de Graciano Sánchez, San Luis Potosí. México.
[5] INIFAP, Campo Experimental Río Bravo, km 61 Carr. Matamoros-Reynosa, Apartado Postal 172, C.P. 88900, Río Bravo, Tamaulipas, México.
* Correspondence: fjesushg@hotmail.com

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ABSTRACT

The objective of the study was to evaluate the seed yield variables *in situ* in *Bouteloua gracilis*, *Bouteloua hirsuta*, *Bouteloua curtipendula*, *Mulhenbergia rigida* and *Schizachyrium scoparium* in La Siberia, Chapingo, Mexico. The study was carried out on wild plants in which the following were recorded: total stems plant^{-1}, number of branches plant^{-1}, number and quantity of seed^{-1}, stem^{-1} and plant^{-1} to later evaluate the percentage of filling and viability. The data were analyzed with GLM of SAS in Completely Random Blocks design and Tukey's test (α=0.05). There was no grass species that exceeded 25 flower stems per plant (P<0.001). *B. gracilis* showed a lower number of inflorescences per plant compared to *B. hirsuta* (P<0.001), however, with a higher number of branches (P<0.001). Seed production per plant (mg) was higher in *M. rigida* (P<0.001; 12902), followed by *B. curtipendula* (2246) and *S. scoparium* (1465). In physical quality of *B. curtipendula* seed, it presented a higher percentage of filling (P<0.001; 17%) and *S. scoparium* greater viability (P<0.001; 78%) and weight of 1000 seeds (P<0.001; 1.52 mg). In La Siberia, *B. curtipendula* and *S. scoparium* were identified as potential grasses to collect seed and then be used for reclamation or rehabilitation.

Keywords: native grasses, seed quality, reclamation lands.

INTRODUCTION

Mexico is the center of origin of many plant species, among them grasses. Dávila *et al.* (2006) report 204 genera and 1182 species, and of these 278 are endemic and 159 are introduced and/or cultivated. In Mexico, the low or null production of seed has


Image by Michaela at Pixabay

reduced the reclamation of agricultural soils to their original vocation (Jurado *et al.*, 2021). Because of this, in semi-arid Mexico, the genus *Bouteloua* has nearly 60 species with broad intraspecific genetic variability (Peterson *et al.*, 2015). The genus *Muhlenbergia* has 145 native species from southern United States and northern Mexico (Rosales *et al.*, 2013). On the other hand, *Schizachyrium scoparium*, of common name little bluestem is a native grass from northern Mexico and southern United States that is very useful in reclamation from agricultural to livestock production (Fu *et al.*, 2004), although studies about this prominent grass species have not been conducted in Mexico.

Knowledge of seed yield variables such as number of flower stems, number of branches per flower stem, number of florets per branch, and therefore percentage of filling, weight of 1000 seeds, and viability (%), allow offering pure viable seed (Rodríguez-Ortega *et al.*, 2021); this is useful for reclamation or rehabilitation. The study of genetic diversity within Mexican grass species, in banderita, has been studied by Morales-Nieto *et al.* (2009), Moreno-Gómez *et al.* (2012), and in navajita by Morales-Nieto *et al.* (2007); however, the in situ study of the yield components to obtain wild seed in banderita, navajita, *Muhlenbergia rigida* and little bluestem have not been reported. Therefore, the study object was to evaluate in situ in navajita [*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths], navajita velluda (*Bouteloua hirsuta* Lag), banderita [*Bouteloua curtipendula* (Michx). Torr.], *Muhlenbergia rigida* (Kunth) Kunth, and little bluestem [*Schizachyrium scoparium* (Michx.) Nash] the following components of the seed yield: number of flower stems, number of branches per stem, amount of seed per plant, percentage of filling, percentage of viability; and in this way, to report fodder species for reclamation or rehabilitation.

MATERIALS AND METHODS

The study was conducted in the experimental area "La Siberia", which contains a surface of 32 ha, in geographical coordinates 19° 28' 18.30" and 19° 27' 41.00" N and 98° 50' 45.92" and 98° 51' 28.17" W, municipality of Texcoco, Estado de México, Mexico. The place is located at 2323 masl with haplic phaeozem and lithosol soil of medium texture and precipitation of 700 mm (INEGI, 2018). The study period covered from the month of June to October 2018. The 60 areas (1 m^2) in La Siberia were limited in three sections within the 32 ha of the total surface, and fixed squares were marked for this according to the species (when inflorescences exerted and could be identified) and a sketch was made so that they could be identified easily later. The variables of the yield components were:

1. Plant height. This consisted in measuring from the base on the ground to the tallest leaf or inflorescence. 2. Number of flower stems per plant (N_FS). The stems with visible inflorescence were counted in the field at the time of harvest. 3. Number of branches per plant (NB). The number of branches was counted in all the stems of navajita and navajita velluda. 4. N_FB. In pectinated grasses the number of florets per branch were counted. 5. Number of branches per flower stem (N_BFS). In non-pectinated grasses, they were counted for three flower stems and multiplied by the total number of stems. Branches were collected along the rachis in the five species and deposited in paper bags that were previously labeled; then, they were placed in a greenhouse with air flow during three

weeks and weighed when the moisture was 12% (Hernández-Guzmán *et al.*, 2021). 6. Seed production per plant (SPP). For this, the weights of the dispersion units of the complete plant were added (mg). 7. Number of plants per m^2 and ha^{-1}. Plants were counted per species in each limited area until each species had exerted inflorescences. 8. Filling (%). Four repetitions were taken of 100 dispersion units of a work sample of 3 g and with the help of clamps and stereoscopic microscope they were dissected, in order to separate plant seeds. 9. Viability (%). The viability was measured with tetrazolium at 0.1%, and for this, 50 cariospsides in good state were obtained from a working sample of 6 g, according to the methodology described by Hernández-Guzmán *et al.* (2021). 10. Pure viable seed ha^{-1} (PVS ha^{-1}). It was determined when multiplying amount of seed per plant × number of plants per ha × percentage of filling × percentage of viability; the result is the amount of effective seed to be considered viable (kg). 11. Weight of 1000 seeds (mg). Eight repetitions were counted from 100 dispersion units and the mean was multiplied by 10 (Hernández-Guzmán *et al.*, 2021).

To consider the moment of harvest for each species, visits were made every seven days, and it was conducted when the inflorescences turned beige color. The dates of harvest were October 21, 2018, for navajita; October 24, 2018, for *M. rigida* and banderita; for navajita velluda, October 26, 2018; and for little bluestem, November 7, 2018. Once the number of branches per flower stem and number of florets per branch had been counted, all the dispersion units of the 60 samples were obtained; they were homogenized and then a sending sample of 30 g was obtained (ISTA, 2012). The data were set up in a completely randomized block design and analyzed with GLM from SAS (2009), and Tukey's test (α=0.05) was used to separate the means. The temperature and precipitation of the place were taken from the meteorological booths of Universidad Autónoma Chapingo, 2 km from the place.

RESULTS AND DISCUSSION

The precipitation and the temperature in the Chapingo region were adequate for growth of the Poaceae plants studied (Figure 1), since they are C_4 plants with summer growth (Gould and Shaw (1992).

Seed yield components

The greatest height in the grass species evaluated was observed in little bluestem (138 cm) and they were all different between one another ($P<0.001$; Table 1), which indicates variability in height and morphology (Alvarez-Lopeztello *et al.*, 2016). In the number of flower stems (N_FS) no difference was found between banderita, *M. rigida* and little bluestem grasses ($P>0.05$), while navajita grass was lower ($P<0.001$; 9.4 stems); however, it is within the range reported by Morales *et al.* (2009), that is, 3 to 186. On the other hand, in our study it averaged 24 in little bluestem and was similar to results by Butler and Briske (1988) in genotypes collected in Texas. Therefore, there is similarity in the same species of North American grass, since they are product of the repopulation of genotypes of Mexican grasses which were lodged by the "eternal" frosts (Quero *et al.*, 2007). In the species of pectinated

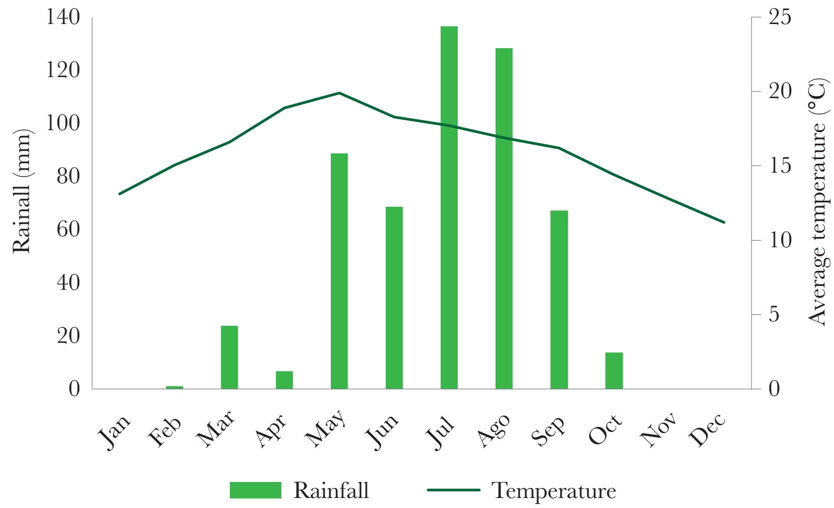

Figure 1. Monthly precipitation (mm) and mean monthly temperature (°C) in 2018 for the zone of Chapingo - Estado de México, Mexico.

Table 1. Average of yield components of 60 plants in five grass species evaluated in the Siberia Field, Texcoco, Estado de México, Mexico. Desert and can be useful in the rehabilitation of degraded soils instead of African grasses.

Species	Hight (cm)	NFS	NTW	NFB	NSS	SPP (mg)
Bouteloua gracilis	77.7 c σ	9.4 b	17.3 b	74.9 a		1 272 b
Bouteloua hirsuta	34.1 e	22.1 a	24.3 a	35.3 b		881 b
Bouteloua curtipendula	64.3 d	22.5 a			99.8 b	2 246 b
Muhlenbergia rigida	114.9 b	22.2 a			552 a	12 902 a
Schizachyrium scoparium	138.0 a	23.4 a			62.0 b	1 465 b
Average	85.8	19.9	20.8	55.1	326.1	3 753
Significancy	***	***	**	***	***	***
SEM	1.14	1.68	1.63	1.12	13.74	725.19

Equal literals per column are statistically similar averages ($P>0.05$). **$P<0.01$; ***$P<0.001$. SEM=Standard error of the mean. NFS=Number of floral stems. NTW=Number of twigs per plant. NFB= Number of florets per twigs. NSS=Number of seeds per floral stem. SPP=Seed production per plant.

grass, navajita velluda showed higher number of flower stems per plant (N_FS; 22.1) and higher number of florets per branch (24.3; $P<0.001$); however, navajita grass presented a higher number of branches per plant ($P<0.001$), which agrees with Gould and Shaw (1992) in the varietal description of each species, and therefore, higher seed production. In the non-pectinated Poaceae species, *M. rigida* stood out for producing higher number of dispersion units (spikelets) per flower stem (N_SFS), generally uniflora, and exceeded banderita and little bluestem by 5.5 and 8.9 times ($P<0.001$), respectively. The highest seed production per plant^{-1} was observed in *M. rigida* (12902 mg) and exceeded banderita by 5.7 times ($P<0.001$) and little blustem by 8.8 times, while banderita grass exceeded navajita by 1.76 times ($P>0.05$); however, these complete dispersion units sometimes do not contain plant seeds, as was mentioned by Quero-Carrillo *et al.* (2016). The species *M.*

rigida has rigid stems and of fibrous aspect (Giraldo-Cañas and Perterson, 2009), so that it is not an appealing species for livestock when it is physiologically mature, although it is a native species from the Chihuahuan.

The amount of plants in La Siberia in the five grass species studied are in low density, and particularly navajita velluda, little bluestem and *M. rigida* with 1 plant m^2 ($P<0.001$), since Quero-Carrillo *et al.* (2016) and Velázquez-Martínez *et al.* (2014) recommend a minimum of 5 plants m^2, which is important for soil retention and fodder production. Because of this, each micro-environment at the level of water reserve and soil nutrients, and their availability for the seedling, there is dominance of one species, known as vegetative types (Hernández-Guzmán *et al.*, 2021). When it comes to filling of the dispersion units (Table 2), differences were observed ($P<0.001$): banderita grass had higher presence of cariopside (17%) and was low in navajita (5.62%) if compared to seed evaluated by Quero-Carrillo *et al.* (2016), from imported seeds from production fields in the south of the United States. Because of this, wild grass plants did not receive the necessary nutrients. According to Zhang *et al.* (2017), in grasses the embryo is formed first and then the endosperm, while in the endosperm, according to Sabelli and Larkins (2009), the syncytium is formed after the double fertilization of the polar nuclei (series of divisions in absence of formation of cell wall and cytokinesis), and lastly the cellularization of the endosperm which includes the formation of principal type cells (transference cells, aleurone, endosperm, and cells that surround the embryo), then mitosis and endo-reduplication, accumulation of storage substances and maturation, which includes dormancy.

In amount of pure viable seed per hectare, differences were obtained in each of the species ($P<0.001$), where *M. rigida* provides more quantity (Table 2). Banderita grass provides 162 kg of VPS which allows recovering approximately 16 ha at a density of 10 kg ha^{-1}, since it is the grass species most studied for semi-arid Mexican zones and seven out of eight varieties are registered in the National Catalogue of Plant Varieties (*Catálogo Nacional de Variedades Vegetales*, CNVV) of the National Service of Seed Inspection and Certification (*Servicio Nacional de Inspección y Certificación de Semillas*, SNICS). On the other hand, in navajita grass (with 1 title of plant breeder) it produced to reconvert or establish 3.7 ha, which is low, although it is a grass resistant to trampling and accepted by livestock

Table 2. Yield variables of four wild grasses in La Siberia, Texcoco, Estado de México, Mexico.

Species	Plants m^2	Plants ha^{-1}	Pollinated (%)	Viability (%)	PVS ha^{-1} (kg)	Weight 1000 seeds (mg)
Bouteloua gracilis	4.0 a $^{\sigma}$	4000 a	5.62 d	52 b	37 c	0.2892 d
Bouteloua hirsuta	1.0 c	1000 c	11.1 b	38 d	37 c	0.6152 c
Bouteloua curtipendula	3.0 b	3000 b	17.0 a	42 c	162 b	0.8635 b
Schizachyrium scoparium	1.0 c	1000 c	5.87 d	78 a	67 bc	1.5211 a
Muhlenbergia rigida	1.0 c	1000 c	7.8 c	34 e	349 a	0.2127 e
Average	3817	2000	10	49	130	0.7003
Significancy	***	***	***	***	***	***
SEM	0.2086	2086	0.45	0.95	27.1	0.00064

Lowercase literals per column are statistically similar means ($P>0.05$). ***$P<0.001$. PVS ha=Pure viable seed ha^{-1}.

(Quero *et al.*, 2017). When it comes to little bluestem, it produces seed to sow 6.7 ha (1.8 times more compared to navajita), and it is a grass species that is appealing to livestock (Butler and Briske, 1988).

Corollary. The rehabilitation of degraded pasturelands of zones with scarce rainfall in Mexico is limited by the knowledge of the functioning of grasslands; therefore, this document contributes information that in natural grasslands there can be grass species that can serve to rehabilitate degraded areas to establish a seedbed with irrigation and as a source of genetic wealth for reclamation or rehabilitation. Because of this, according to Jurado-Guerra *et al.* (2021), in Mexico grasslands are over-grazed in 95% and in shrubs 70%, which limits the production of the cow-calf system. Thus, the application of laws on conservation of resources and sustainable use of ecosystems is urgent, where the semi-arid zones are the most fragile (Hernández-Guzmán *et al.*, 2021).

CONCLUSIONS

In the grassland ecosystem of La Siberia, Texcoco, Estado de México, in the five Poaceae species studied, banderita (*Bouteloua curtipendula*) and little bluestem (*Schizachyrium scoparium*) grasses stand out because they are the most appealing to the livestock and due to their good seed production and quality, and must be considered for reclamation and rehabilitation of pasturelands.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Technology use and profitability analysis of the production system of tejocote (*Crataegus* spp.) in Sierra Nevada, Puebla

Núñez-Ramírez Ramón[1]; Jaramillo-Villanueva José Luis[1]; Carranza-Cerda Ignacio[1]; Barrera-Rodríguez Ariadna I.[1*]; Huerta de la Peña Arturo[2]

[1] Colegio de Postgraduados Campus Puebla, Puebla, México, C.P. 72760.
[2] Universidad Autónoma Chapingo. Chapingo, Estado de México, México, C.P. 56230.
* Correspondence: ariadna.barrera@gmail.com

ABSTRACT

Objective: The objective of this research was to analyze the use of agricultural technology and the profitability of the tejocote (*Crataegus* spp.) production system and its explanatory factors, in order to identify actions to increase the income of fruit growers.

Design/methodology/approach: Two patterns were used (producers who carry out good phytosanitary practices and producers who export). A stratified sample of 90 fruit growers was obtained, with 95% reliability and 10% accuracy. Subsequently, a survey was carried out and the profitability of tejocote production was calculated with the data. In addition, the explanatory factors of profitability were identified using a multiple regression model.

Results: Profitability, measured with the Benefit-Cost indicator, ranged from 0.13 to 2.38, and an average of 0.84. In the scenario of not accounting for family labor or depreciation of infrastructure, a Benefit-Cost Ratio from 0.35 to 6.37 is achieved, with an average of 1.90. The use of technology, measured by the technological index (TI) in the cultivation of tejocote was significant to improve profitability. The average profitability of the high TI stratum is different from the average profitability of the medium and low TI strata. In addition, the explanatory factors of profitability that were significant ($p \leq 0.05$) were technological index, phytosanitary control, training, schooling and size of the plantation.

Study limitations: Most producers do not keep a record of production activities and costs. Likewise, in the analysis of economic profitability it is difficult to assess indirect benefits, intangible benefits and externalities.

Findings/Conclusions: It was found that the tejocote activity is profitable for producers who use more technology and have a greater number of trees in production. The variables that most impact profitability were use of technology, phytosanitary control, size of the plantation, and training.

Keywords: Small-scale producers, production system, financial and economic profitability, benefit-cost ratio.

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INTRODUCTION

Tejocote (*Crataegus* spp.) (Rosaceae) is a native fruit from Mexico that belongs, subfamily of Pomoideae and genus *Crataegus*. Since pre-Hispanic times, tejocote has been used by different indigenous groups in Mexico, which collected their fruits. Then, trees of this type were planted in their gardens, and with the arrival of the Spanish, tejocotes were selected and planted in commercial orchards (Núñez *et al.*, 2012).

Image by wasi1370 at Pixabay

In Mexico, the most important use is as fresh fruit primarily related with culture. For example, in the offerings of the "Todos Santos" festivity, and for use in "piñatas", punch, and tejocote liquor during December Posadas, since it contributes calories to the human body that are necessary due to low temperatures (Nieto *et al.*, 2008).

In Mexico there are 945.37 hectares planted with tejocote and 5,521.82 tons were harvested in 2019 with a production value of 1199.8 thousand USD. The average yield is 5.95 t ha^{-1} per hectare and the price was 217.29 USD per ton. In Puebla there are 897.6 hectares planted with tejocote and 5,336.94 tons were harvested in 2019 with a production value of 1132.91 thousand USD. The average yield is 6.04 tons per hectare and the price was 212.28 USD per ton (SIAP, 2019).

As can be seen from the above paragraph, 97% of the national production comes from Puebla. There are 29 producing municipalities and the following stand out because of their surface planted: Calpan (15%), Huejotzingo (13%), Chiautzingo (12%), Soltepec (9%), San Salvador El Verde (7%), Tlahuapan (7%) and Domingo Arenas (7%) (SIAP, 2019). During the year 2020, 990 tons of tejocote were exported to the United States of America (SAGARPA *et al.*, 2021). According to the National Service for Agrifood Health, Safety and Quality (Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria, SENASICA), out of the 10 exporting companies of tejocote, nine are found in Puebla.

From the data presented, it can be stated that Puebla is the main tejocote producer in the country, and that this fruit tree has high economic importance as a source of income for fruit producers in the state. However, there are several problems that families have to deal with, from the productive process to the commercialization of the product, such as: small farms with low technology, high production costs, and scarce resources to invest; adverse climate conditions such as drought, hail and frosts; pests and diseases; as well as low yields, low quality of the product, and unfavorable purchase prices (Núñez *et al.*, 2012).

Several studies have been conducted in tejocote which refer to pests such as fruit fly and fruit borer; also about morphological and phenotypical variability of trees and fruits; biochemical analyses and studies on physiology (López *et al.*, 2008; Nieto *et al.*, 2008; Muñiz, 2011; Núñez *et al.*, 2012; García *et al.*, 2013; Rosas *et al.*, 2017). However, despite these advances, the economic information of this production system is unknown, as well as the factors that influence profitability. Therefore, if the importance of this production system for the state of Puebla is considered, as well as the problems that producers face, it is necessary to carry out an analysis of financial and economic profitability of the tejocote production system, with the aim of identifying actions to increase the returns of producing families, primarily the poorest. Likewise, the hypothesis set out is that the tejocote production system is profitable from the economic and financial point of view. This study was justified because actions were identified through the economic analysis which contributes to a higher profitability for small-scale tejocote producers. Also, this study is relevant to the degree that it will contribute to the construction of strategies to improve the tejocote production system in the Sierra Nevada region of Puebla, Mexico.

MATERIALS AND METHODS

The study was conducted in the Sierra Nevada region in Valle de Puebla, in the municipalities of Huejotzingo, Calpan, Tlahuapan, Chiautzingo and San Salvador el Verde. The predominant climate is temperate sub-humid with summer rains, the average temperature varies between 12 and 16 °C, rainfall ranges from 600 to 800 mm/year, and the altitude between 2,000 and 2,300 masl (Gutiérrez *et al*., 2003). In the study zone, livestock production and agriculture are important economic activities. Mixed production systems are found that involve fruit trees of temperate climate (including tejocote) and annual crops, primarily basic grains such as maize and bean (Mendoza *et al*., 2010). Regarding the condition of poverty, the municipality of Huejotzingo presents between 50% and 75% of population in poverty. In contrast, Calpan, Tlahuapan and Chiautzingo have between 75% and 100% of their population in poverty (CONEVAL, 2021).

In the calculation of the sample of producers to interview, the database of producers that carry out good phytosanitary control practices was used, from the State Committee of Plant Health in the State of Puebla (*Comité Estatal de Sanidad Vegetal del Estado de Puebla*, CESAVEP) and the database of producers who exported tejocote from the National Service of Agrifood Health, Safety and Quality (SENASICA). In total, 414 different producers were quantified and based on this sampling universe, a simple probabilistic sample (random) was calculated, with reliability of 95%, accuracy of 10%, and considering maximum variance, a sample of 90 producers to be interviewed was obtained.

Profitability of the tejocote production system

The financial profitability of the production system was analyzed in this study. The financial evaluation assesses, through market prices, the direct benefits and costs of an investment project; that is, the return obtained by the private investor. For their part, the economic evaluation determines the benefits and costs produced by individuals who belong to a system, society or country, evaluating their investment resources at the prices that they really cost, under a perspective of shared welfare (FIRA, 2011).

Benefit - Cost Ratio Calculation (B/C)

The Benefit-Cost Ration was considered in the study, which calculates the present values for each of the years, both of updated costs (C) and updated benefits (V), and adding these they are divided using the following formula:

$$B/C = \frac{\sum_{i=0}^{n} \frac{B_i}{(1+i)^n}}{\sum_{i=0}^{n} \frac{C}{(1+i)^n}}$$

Where: B_i is the value of the benefits; $(1+i)^{\mathrm{n}}$ is the updating factor; and C_i are the costs.

The result obtained in the previous operation indicates the profit obtained for each peso invested. If the B/C ratio is higher than 1, it means that the benefits exceed the costs and the greater the result, the benefits will be higher; for example, if the result was 1.5, it means that 1.5 pesos of benefits are obtained for each peso spent (Morales and Salinas, 2010).

The profitability was also analyzed considering the producers that use different levels of technology use, represented by the technological index (TI), measured in a scale of zero to one. The TI was constructed considering seven technological components applied to the crop, which are: number of productive assets, fertilization, producer's abilities, pruning, irrigation, pest control, and plantation density. The value of the technological index was obtained by adding the value of all the variables mentioned and then dividing the result by seven.

RESULTS AND DISCUSSION

The average age of the producers was 54 years and a schooling level of 7.3 years (secondary school). On average, they own 0.96 ha, 267 trees, have a production of 8.44 tons annually, a yield of 9.6 t ha^{-1} of tejocote, and average technology use index of 0.57 (Table 1). These characteristics indicate a type of small-scale fruit production of family type. The technological components with lowest values were irrigation (0.30) and pruning (0.31), then the use of machinery and equipment (0.56), fertilization (0.63), and pest control (0.71), and the highest was training of the producer (0.84).

The average profitability, represented by the Benefit-Cost Ratio was 0.84, and median of 0.82, with a minimum of 0.13 and maximum of 2.38. However, it is important to mention that 68% of the producers interviewed obtained a B/C ratio with a value of 1.0 or less (not profitable), due to the strong hail storms, drought, low market prices, and high annual costs from the depreciation of assets (machinery and infrastructure). These results are close to those reported in the technological package of the tejocote crop presented by Ríos (2018), who calculated a B/C ratio of 0.71.

Within the cost structure, the fixed costs represent 30% and the variables 70%, and within the latter, the workforce represented 56%. The establishment of the crop (preparation of the soil, fertilizer and workdays) had a cost, on average, of 842.85 USD (Table 2). Among the variable costs (consumables, irrigation, workforce, fuel and payment of services), and after

Table 1. Summary of sociodemographic and technological variables.

Variables	Number of observations	Media	Standard deviation	Minimum	Maximum
Age	90	53.53	12.43	21	85
Schooling (years)	90	7.26	4.03	0	16
Land (ha)	87	0.96	0.67	0.25	4
Number of trees	90	267	245.19	30	1300
Production (ton)	89	8.44	6.32	0.61	28
Yield (ton ha^{-1})	87	9.6	5.86	0.61	31.7
Technological index	90	0.58	0.1543	0.271	0.931

Table 2. Principal concepts of production costs of Tejocote (USD).

Cost component	N	Minimum	Maximum	Media	Standard deviation
Establising costs	90	125.05	3547.5	842.845	688.91
Depreciation	90	159.5	5400	491	1037.425
Fixed costs	90	308.9	5782.1	1283.03	1222.045
Labour cost	89	180	17598	1813.35	1267.505
Variable cost	90	215.35	40217	3146.9	4457.655
Total cost	90	524.25	45999.1	4429.95	2945.005
Sales income	90	258.75	46710	3727.565	5677.375

subtracting the fixed and variable costs from the income from the tejocote sales, returns were obtained from −7268.0 USD to 9604.3 USD. Therefore, there are production units that are having losses.

According to the National Council of Social Development Policy Evaluation (*Consejo Nacional de Evaluación de la Política de Desarrollo Social*, CONEVAL), the monthly value per person of the basic food basket and non-food rural basket of the month of July 2021 was 111.93 USD. Therefore, for most of the tejocote producers, the return obtained is not enough to satisfy family needs.

Benefit-Cost Ratio and returns obtained (without considering family workforce or expenditure from depreciation of high-value assets)

In a scenario where the costs for family workforce or depreciation of infrastructure were not considered, a B/C ratio was achieved from 0.35 to 6.37, an average of 1.90 and median of 1.73. Thus, 7.78% of the producers obtain a B/C ratio with value of 1.0 or less (non-profitable) and 92.22% a higher value at 1.0 (profitable).

Level of technology use and yields

The level of technology use in the tejocote production process is a factor that showed strong association with the level of profitability. The analysis of variance revealed that the level of technology use, represented by the "low" and "medium" technological index (TI), belongs to the same group, which means that the group of producers with "low" and "medium" TI have a tejocote mean yield (ton/ha) that is statistically equal. Instead, "low" TI and "high" TI have different means (Table 3). These results are similar to those reported by Vázquez *et al.* (2020) in a study carried out in Guerrero, Mexico, in lime cultivation.

Explicative factors of profitability

The explicative factors of the profitability of tejocote are shown in Table 4. Regarding the goodness of fit of the model, taking independent variables together, they explain 74% of the behavior of profitability of tejocote production. The values of the F(14.2) statistics reject the null hypothesis that the population value of R^2 is zero. Therefore, there is a significant linear relation ($p \leq 0.05$). Values of colinearity lower than 10 denote absence of correlation between explicative variables.

Table 4. Explicative factors of profitability of tejocote.

Variables	Coefficients	Typical error	t-value	Collineality
(Constante)	−0.185	0.259	−0.71	−0.71
Age	0.001	0.003	0.41	0.33
Training	0.431	0.137	3.14	3.15
Safety	0.573	0.165	3.47	3.47
Familiar labour	−0.107	0.126	−0.85	−0.85
Organization	0.141	0.08	1.75	1.76
Monetary transfers	−0.062	0.074	−0.84	−0.84
Technological index	0.795	0.242	3.29	3.29
Technical assistance	0.05	0.077	0.64	0.65
Schooling	0.299	0.142	1.97	2.11
Size of farm	0.249	0.124	1.6	2.01
R-squared/Adjusted		0.74-0.725		
F-value		14.2		
Durwin-Watson		2.08		

In order of importance, the technological index, phytosanitary control, training, schooling and size of the plantation are variables that are significant ($p \leq 0.05$) to explain the behavior of the profitability of the crop. The literature review performed did not result in any previous study that has studied the explicative factors of the profitability of this crop, so what was found in this study is discussed with what has been reported in the literature for other fruit trees.

The technological index represents the incorporation of the practices of management, nutrition and phytosanitary control to the crop. The positive and significant effect of the use of agricultural technology on the profitability of tejocote was also reported by Ma and Abdulai (2019) in a study on adoption of technology in apple growing in China, by Wambua *et al.* (2019) in a study about coffee in Kenya, and by Vásquez *et al.* (2020) in a study about lime in the state of Guerrero.

Schooling was reported as a variable associated to the incorporation of higher levels of technology, yields and profitability. In this regard, Xu *et al.* (2009) report that in a study conducted in Zambia, schooling had a positive effect on profitability. The size of the agriculture and livestock production units also has a positive effect on profitability, and therefore on the income of producers. This result refers to producers with more surface planted or higher number of producing trees who obtain higher profitability, by having lower unitary costs (Bravo-Monroy *et al.*, 2016).

CONCLUSIONS

The tejocote production system in the Sierra Nevada region of Puebla is profitable from the financial point of view for producers who use more technology and have a higher number of producing trees. The workforce represents the most important cost in the annual production process. Of all the workdays required, the activity of harvesting, selecting and packaging is the one that generates the highest cost. Therefore, it is important to increase the level of technology, primarily the investment in machinery and equipment for the application of agrichemicals, weed control, land preparation, and fruit selection, as well as the design of orchards with formation systems that keep trees that are small and with shapes that ease harvesting, since these actions significantly decrease the number of workdays used.

The use of technology is directly related to higher profitability. The mean of profitability in the strata with low and medium technological index belongs to the same group, while the stratum of producers with high and low TI has a significant difference in the mean. Finally, the explicative variables of profitability were technological index, phytosanitary control, training, schooling and plantation size. This is why these variables could be used in the design of a strategy that tends to improve the yields and the profitability of tejocote in the study region.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Use of agroindustrial waste to obtain cellulose from oil palm bagasse (*Elaeis guinnensis*)

Tirado-Vasquez, Alfredo[1]; **Cadenas-González, María T.**[2*]; **Bolio-López, Gloria I.**[1]; **Hernández-Córdova, Roberto**[3]; **Leo-Avelino, Gabriela**[2]; **Ramos-Valencia, José J.**[2]; **Castañeda-Castañeda, Carlos B.**[4]

[1] Division Académica de Ciencias Básicas e Ingeniería. Universidad Popular de la Chontalpa. Km. 2. Carr. Cárdenas-Huimanguillo, Cárdenas, Tabasco, México. 86500.

[2] Gerencia de Procesos. Centro de Tecnología Avanzada (CIATEQ, AC.), C. 23 de agosto 213. Col. Jesús García. Villahermosa, Tabasco, México. 86040.

[3] Centro de Investigación de Ciencia y Tecnología Aplicada de Tabasco (CICTAT). Universidad Juárez Autónoma de Tabasco, DACB, Km.1 carretera Cunduacán-Jalpa de Méndez, Cunduacán, Tabasco, México. 86690

[4] Direccion de Ingeniería y Construcción de Plantas. Centro de Tecnología Avanzada (CIATEQ, A.C.), Av. del Retablo 150 Col. Constituyentes FOVISSSTE. Querétaro, Qro, México. 76150.

* Correspondence: teresa.cadena@ciateq.mx

ABSTRACT

Objective: To obtain cellulose from oil palm agroindustrial waste that meets standard physicochemical characteristics to produce value-added products.

Design/methodology/approach: Bagasse fibers from the palm agroindustry were used to obtain cellulose, by means of the acid – alkaline hydrolysis methodology. The samples obtained in each stage and the cellulose obtained were characterized by the Fourier Transform Infrared Spectroscopy (FTIR) technique and thermogravimetric analysis (TGA).

Results: The final characterized product presented a band corresponding to 1370 cm^{-1} which is a characteristic value of cellulose. The peak at 1731 cm^{-1} is related to C=O bonds of unconjugated ketones present in hemicellulose. A major thermal event for treated fiber near 355 °C and the high residual mass indicate a good chemical treatment for hemicellulose and lignin elimination.

Study limitations/implications: A fiber yield of 39.3% cellulose was obtained during the process from oil palm bagasse.

Findings/conclusions: Obtaining cellulose from a highly polluting residue such as palm bagasse and with high production figures in our state, makes it a potential for use to generate biopolymers in combination with natural polysaccharides, providing sustainable benefits and economic impact and promoting sustainable development by replacing conventional fossil plastics, in addition to obtaining value-added products for the same agribusiness and in sectors such as the food industry.

Keywords: Agribusiness, Oil Palm Bagasse, Pulp, Cellulose.

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INTRODUCTION

Oil palm production in Mexico has increased by 60% in the last 5 years and it is developed mainly in states located in the south-southeast of the country; in Tabasco, it is found in the regions Sierra and Center, with a surface of 26,718 Ha, which makes it

Image by tk tan at Pixabay

the fourth agribusiness in the state (SIAP, 2020). Oil production generates byproducts of technical and economic interest such as husks, fibers resulting from fruit pressing, pits, and ash. Around 1,1 t of fibers generated per ton of oil produced could be used as prime material for industrial and artisanal products, and to elaborate biocompounds, which reduces the use of synthetic polymers, thus obtaining higher degradability, lower toxicity, better mechanical properties, and thermal resistance (Shinoj *et al*., 2011). Presently the abundant amount of empty oil palm bunches has caused environmental problems, such as combustion and generation of fungi in the accumulated residues. The disposal of residues generated during the oil extraction process represents a great challenge, since these ought to be used or have some treatment before being eliminated. Considering the above, this study presents an alternative for the use of these agroindustrial residues. The purpose consists in obtaining cellulose from oil palm bagasse (*Elaeis guineensis*), contributing to the decrease of agroindustrial waste, in addition to obtaining an added-value product.

MATERIALS AND METHODS

The biological material used (palm bagasse) was supplied by an oil palm agribusiness located in Tacotalpa, Tabasco, Mexico. This material was found with excess moisture and dirt, so it was washed with running water and sun-dried at environmental temperature for 24 h. The material was selected after drying (Figure 1A), since the bagasse is mixed with other fibers and residues generated during this agroindustrial process. The clean and dry bagasse was ground in an industrial blender brand Veca International model LI-17A, with the aim of reducing the fiber chains to an approximate size of 4 cm (Figure 1B).

Cellulose extraction

A pre-treatment on the fiber began once the bagasse was dried and ground, with the aim of eliminating pectins and resins present. The methodology used was proposed by Bolio *et al.* (2011) through the Cazaurang method, conducted in 5 stages: pre-treatment, hydrolysis, chlorination, alkaline extraction and blanching. The pre-treatment consisted in leaving the fiber to rest in a NaOH solution at 10% (p/v) at environmental temperature and

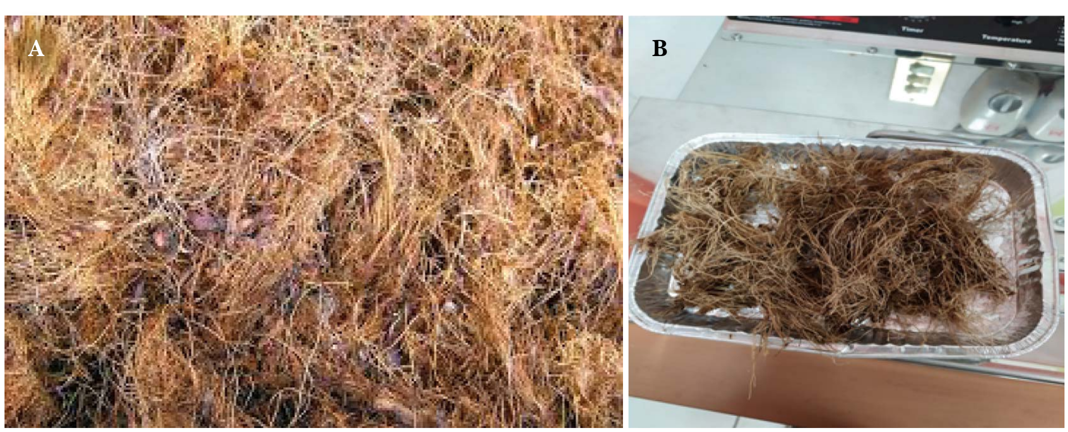

Figure 1. Prime material (oil palm bagasse).

in mechanical agitation for 120 min (Figure 2A), and the resulting fiber was washed with running water until reaching a neutral pH.

Acid hydrolysis. During this stage, access to reagents in the fiber to remove amorphous regions was eased, and the pre-treated fiber was placed in a H_2SO_4 solution at 0.4% maintaining a temperature of 90 °C for 60 min with constant agitation; after this time the fiber was washed until obtaining a neutral pH (Figure 2B).

Chlorination. The resulting fiber of the prior step was incorporated to a NaClO solution at 3.5%, at a temperature of 40 °C and with constant agitation for 10 min. After this time, the fiber was washed with running water until obtaining a pH of 7.0.

Alkaline extraction. This stage has the objective of solubilizing the hemicellulose and reducing the size of the cellulose chains. A NaOH solution at 20% (p/v) was incorporated to the product from the chlorination stage during 10 min and kept with manual agitation, and then with agitation at 150 RPM for 50 min (Figure 2C). After this time, the fiber was washed with running water until obtaining a pH of 7.0.

Blanching. In this step the chromophore groups and residual lignin were eliminated, the previous fiber was taken to a solution of NaClO 0.5% with continuous agitation at 150 RPM in a Mixer Model BDC1850 during 60 min (Figure 2d). Finally, the fiber was washed until reaching a neutral pH and left to dry at room temperature for 24 h and then

Figure 2. Cellulose extraction process from oil palm bagasse. A: Bagasse weighing; B: Pre-treatment; C: Drying in conventional furnace; D: Acid hydrolysis; E: Drying; F: Chlorination; G: Drying; H-I: Alkaline extraction; J: Blanching; K: Rest; L: Dry fiber/weighing; M: Cellulose.

in a drying furnace at 60 °C for 24 h (Figure 2E). Each of the samples was stored in airtight bags for their later characterization.

Fourier Transform Infrared Spectroscopy (FTIR)

To determine the functional groups present in the samples obtained in each of the stages, a Fournier Transform Infrared Spectrophotometer (FTIR) was used, brand Thermo Scientific, model Nicolet™ Is™ 50 with ATR module and diamond crystal, using measurement intervals of 100 and 32 scans.

Thermogravimetric Analysis (TGA)

This test was conducted in a Setaram Labsys EVO 1110 equipment. The sample between 6.0-8.5 mg was placed in a platinum crucible and warmed from environmental temperature to 650 °C with a slope of 10 °C/min. The dragging gas used was argon at 20 psi.

RESULTS AND DISCUSSION

Cellulose extraction

During the process, a fiber yield of 39.3% of cellulose was obtained from the oil palm bagasse. Table 1 shows the cellulose fiber yield (%), which is lower compared to: banana pseudo-stem residues with yield of 66% (Bolio *et al.*, 2011), sugarcane bagasse with 48% (López-Martínez *et al.*, 2016), and the one found in platanillo (*Heliconia latispatha*) with 46% of cellulose obtained (García Fajardo, 2015). On the other hand, the yield obtained from the palm bagasse is higher than 29.3% obtained in residues of pineapple crown (Presenda *et al.*, 2020) and the 34% of final cellulose yield from sugarcane hay by Bolio *et al.* (2017).

FTIR Analysis

Figure 3 shows the infrared spectrum of oil palm bagasse and cellulose obtained. The bagasse sample presented characteristic lignin bands such as the peak and 1720 cm^{-1} related with carboxyl group bonds, which was eliminated during the process of cellulose extraction (Figure 3). Meanwhile, the peak of 1054 cm^{-1} is characteristic of the C-O bond (Contreras *et al.*, 2010). Moran *et al.* (2008) mentions that the peak of 900 cm^{-1} corresponds to C-H groups of aromatic hydrogens from lignin, which decrease their intensity corroborating that the process applied in this study allows removing most of the lignin in the cellulose extracted and at 1630 cm^{-1} the band corresponding to adsorbed water.

Table 1. Cellulose yield obtained.

Residue	Cellulose yield (%)	Reference
Palm bagasse	39.3	Present study
Cane Straw (*Saccharum* spp.)	34	Bolio *et al.* (2017)
Sugarcane bagasse (*Saccharum* spp.)	48	López-Martínez *et al.* (2016)
Platain (*Musa* sp.)	66	Bolio *et al.* (2011)
Platanillo (*Musa* sp.)	46	García Fajardo (2015)

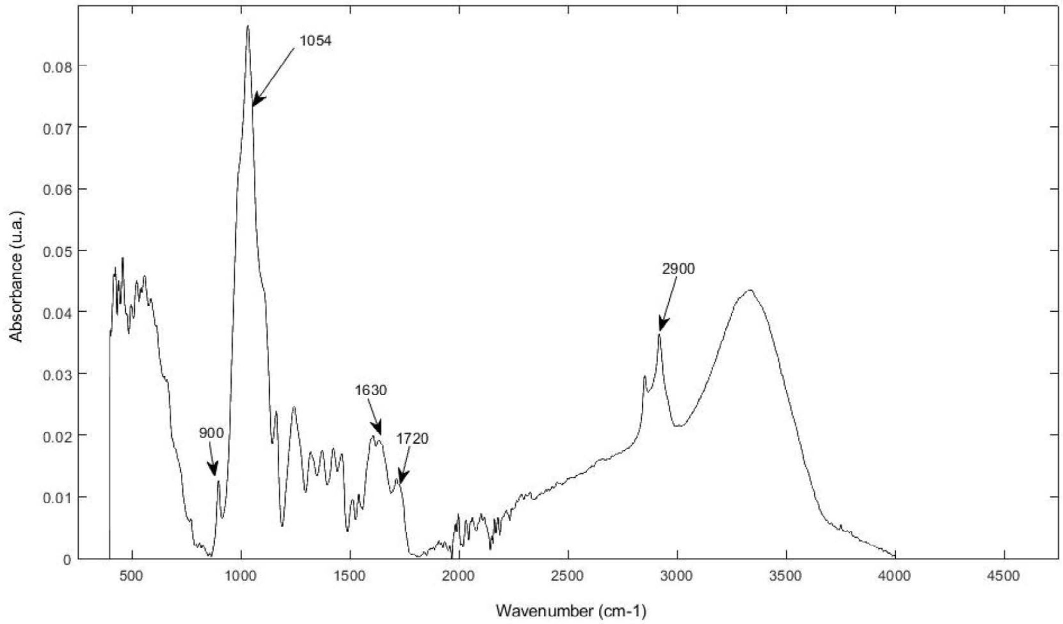

Figure 3. FTIR of oil palm bagasse.

The spectrum corresponding to cellulose (Figure 4) shows a band that corresponds to 1370 cm^{-1} which is characteristic of cellulose. The peak at 1731 cm^{-1} is related with C=O bonds of unconjugated ketones present in hemicellulose (Asfanas'ev *et al.*, 2007). Bands close to 3270 cm^{-1} and 710 cm^{-1} are observed, corresponding to contributions from cellulose Iσ(Boisset *et al.*, 1999). Likewise, the sample is constituted by a compound of bonds that belong to ethers present in the band 1020 cm^{-1} (Contreras, 2010).

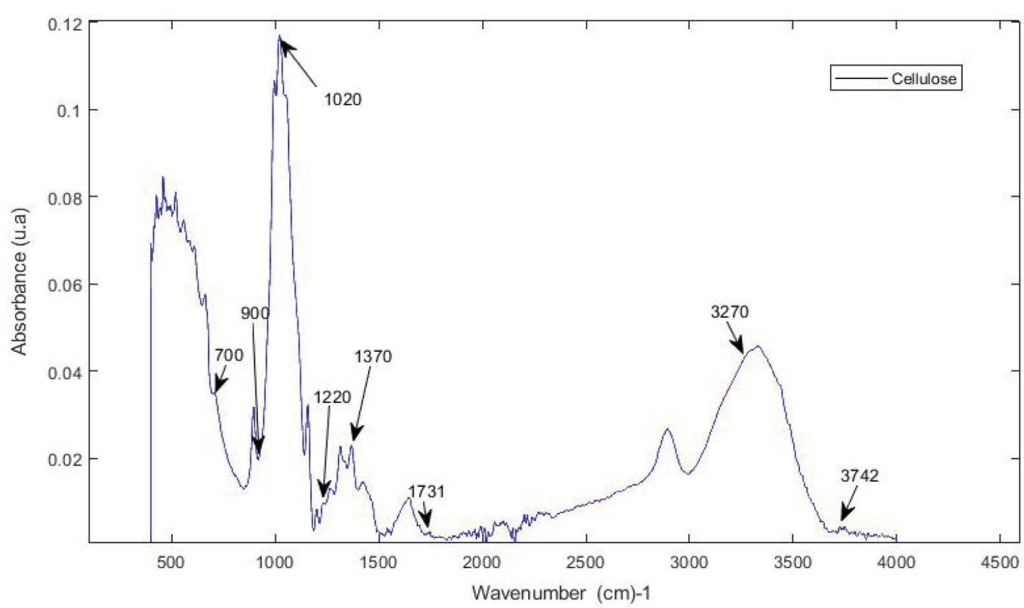

Figure 4. FTIR of cellulose obtained from oil palm.

Thermogravimetric Analysis (TGA)

The thermogravimetric analysis was used to evaluate the thermal stability of the samples and to determine the quality of the cellulose obtained. The results are shown in Figure 5 and in Table 2. The TGA curve measures the loss of mass in function of temperature, while the DTGA curve corresponds to the one derived from the loss of mass, which indicates the decomposition speed of the sample.

It is observed that the bagasse and cellulose samples of oil palm present a mass loss of around 3.5% in 120 °C, corresponding to loss of moisture. The bagasse begins its decomposition at around 246.5 °C, while the cellulose starts at 286.81 °C. The delay in the cellulose decomposition is because the resin compounds, the hemicellulose and most of the lignin have been previously eliminated. This is proven when observing the first event of the DTGA curve from the bagasse, which begins at 239.3 °C and ends at 306.0 °C with a peak at 293.9 °C, corresponding to the thermal decomposition of hemicellulose and pectin compounds, in addition to the rupture of glycosidic bonds of cellulose; on the contrary, in the DTGA of the treated fiber it is not possible to observe this event, because of the elimination of these compounds during the alkaline treatment. The greatest event in thermal decomposition takes place at 349.6 °C and 355.5 °C for bagasse and cellulose, respectively, which corresponds to the thermal decomposition of αcellulose (Khalili, 2008; Kumneadklang, 2019). The delay in temperature of this peak shows that the fiber

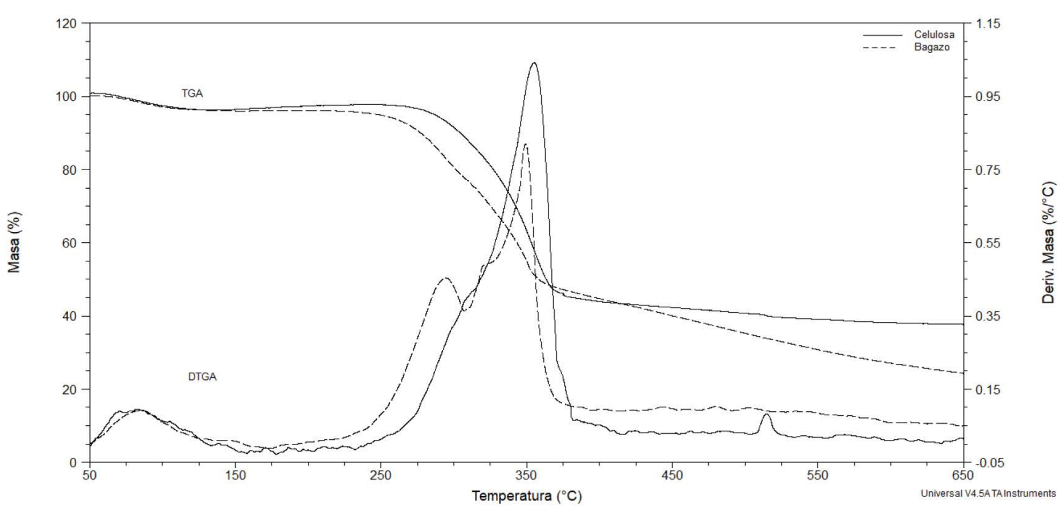

Figure 5. Thermogravimetric analysis of bagasse and oil palm cellulose.

Table 2. Thermal properties of the bagasse and cellulose.

Variable	Bagasse	Cellulose
T_{onset} (°C)	263.6	288.1
Residual mass (%) a 650 °C	24.3	37.7
T_{max} (°C)	349.5	355.5

T_{onset} (°C), Onset of thermal decomposition.
T_{max} (°C), Maximum thermal decomposition temperature.

treated has a higher thermal stability than bagasse, indicating that the hemicellulosic and lignocellulosic compounds have been mostly eliminated. A small decomposition event from the cellulose sample takes place at 515.1 °C, possibly due to the volatilization of short-chain lignin residues (Okoroigwe & Saffron, 2012). The high content of ash at the end of the test is an indicator of a correct treatment of the fiber and from which cellulose of good quality has been obtained.

CONCLUSIONS

This study showed the possibility of obtaining high-quality cellulose at the laboratory level from oil palm agroindustrial residues, applying acid hydrolysis and blanching to the cellulose fibers, and corroborating with infrared analysis and thermogravimetric analysis. These were performed both for the palm bagasse fiber and the cellulose obtained, where a decrease was observed primarily in the intensity of the peaks corresponding to the functional groups of aromatic lignin rings, and in addition, the thermal events characteristic of hemicellulose and lignin were not observed in the cellulose obtained, indicating that they were removed in greater proportion with the chemical procedures applied.

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Proximal and mineral composition analysis of castilla squash seeds (*C. moschata*), green pea (*P. sativum*) and green bean (*P. vulgaris*) for use in 4.0 Agribusiness

Martínez-Betancourt Selena R.[1]**; López- Martínez Laura A.**[2*]**; Rössel-Kipping Erich D.**[1]**; Loera-Alvarado G.**[1]**; Ortiz-Laurel H.**[3]

[1] Colegio de Postgraduados. Programa innovación en manejo de recursos naturales. Iturbide 73, Salinas de Hidalgo., S.L.P. México. C.P. 78600.
[2] Coordinación Académica Región Altiplano Oeste de la Universidad Autónoma de San Luis Potosí. Carretera Salinas-Santo Domingo 200, Salinas de Hidalgo., S.L.P. México. C.P. 78600.
[3] Colegio de Postgraduados. km. 348, Carretera Fed. Córdoba-Veracruz, Congregación Manuel León, Amatlán de los Reyes, Veracruz. México. C.P. 94946.
* Correspondence: araceli.lopez@uaslp.mx

ABSTRACT

Objective: To determine the proximal and mineral composition analysis of of castilla squash seeds (*C. moschata*), green pea (*P. sativum*) and pinto saltillo green bean (*P. vulgaris*).

Design/methodology/approach: The contents of macronutrients (carbohydrates, fats, crude fiber, protein, and ash) and micronutrients (minerals) of the evaluated species were determined using the AOAC standard procedures, and the quantification of minerals was performed by mass spectrometry with inductively coupled plasma. A variance analysis and means comparison were performed with Tukey's test (α=0.05).

Results: The squash seeds with shells contain 291,500 ppm of protein, 417,000 ppm of fat, 66,700 ppm of carbohydrates, 134,000 ppm of crude fiber, 972,319.678 ppm of phosphorus, 3,380.09158 ppm of potassium and 3,183.2744 ppm of magnesium. The whole pea pod has 230,600 ppm of protein, 17,200 ppm of fat, 456,000 ppm of carbohydrates, 220,800 ppm of crude fiber, 5,438.18,991 ppm of phosphorus, 7,349.23753 ppm of potassium and 1,719.56882 ppm of magnesium. The whole green bean pods had a content of protein (185100 ppm), fat (2540 ppm), carbohydrates (377500 ppm), fiber (208000 ppm), phosphorus (6068.44661 ppm), potassium (15626.9991 ppm) and magnesium (17222.16567 ppm).

Study limitations/implications: Studies on the chemical characteristics of other agroindustrial residues should be carried out.

Findings/conclusions: The foods analyzed have adequate characteristics to satisfy the demands with respect to some macronutrients and micronutrients. They can optimize the availability of nutrients in the generation of natural or synthetic foods that can change the future.

Keywords: Chemical content, *Cucurbita moschata*, *Pisum sativum*, *Phaseolus vulgaris*.

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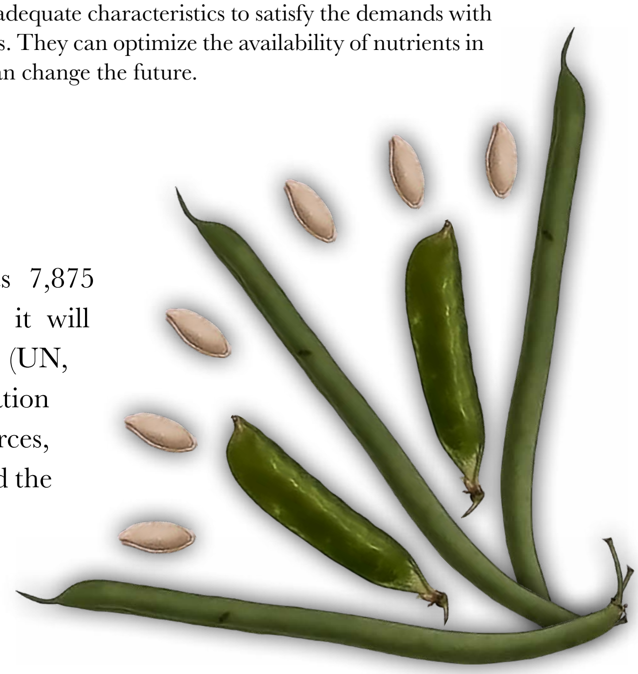
INTRODUCTION

In 2021, worldwide population was 7,875 million people and it is expected that it will increase another 2,000 million by 2050 (UN, 2021). This excessive growth of the population is demanding more and more resources, which affects the planet's biodiversity, and the health and wellbeing of the population (Labrador, 2006). Therefore, the



need arises to find new sources of good quality food which are also accessible and low cost, as well as to adapt new technologies for the management of raw materials, input downsizing in the process, and better use of the quality and quantity of food and non-food products.

Mexico has a great diversity of valuable food resources, and studying them provides information for an efficient use and to continue with sowing and consumption of traditional foods in order to counteract health problems, food security, to increase the country's economy, exploit industrial purposes, and generate new benefits with the elaboration of synthetic foods. Also, it is important to study non-conventional or non-traditional foods such as agro-industrial residues, which, in spite of not representing the main value of the transformation can be a complement for raw material of another product (Saval, 2012), contributing as an option to the grave worldwide food problem.

These residues can provide a nutritional balance in terms of macro and micronutrients, since, in order to increase food quality, crops that decrease vitamin and mineral deficiencies must be included in the population's diet as a sustainable long-term solution (Pachón, 2010).

The current lifestyle promotes inadequate dietary habits such as the consumption of foods of low nutritional quality. In addition to this, many foods lose a great part of their minerals through the industrial refining process, which is why their deficiencies are common among the population. However, minerals are essential for a broad range of metabolic functions in the human body and some of them participate directly or indirectly in many functions of the organism (Velasco-Lavín, 2016).

Legume pods and oilseed seeds can contribute to counteract health and food security problems; nevertheless, these are generally only used in a traditional way (as seeds, canned or frozen goods) and not as raw materials in the transformation of new flour-based products with added value. On the other hand, information on the content of mineral elements in foods is insufficient.

International agencies recommend daily intakes of calcium, magnesium, zinc, iodine, iron and selenium to the general population. For example, in children from one to eight years of age the recommended intake of calcium is 500 to 800 mg day^{-1}, magnesium 80 to 130 mg day^{-1}, zinc 3 to 5 mg day^{-1}, iron 7 to 10 mg day^{-1}, phosphorus 460 to 500 mg day^{-1}, iodine 90 μg day^{-1}, copper 340 to 440 μg day^{-1} and selenium 20 to 30 μg day^{-1} (WHO and FAO, 2004). Deficiency problems in protein, essential fatty acids, fiber, carbohydrates, vitamins and minerals in the diet get more and more complicated because of the lack of information about the chemical content, and this depends on many factors such as type of food, genetic origin, geographic location and agricultural procedure implemented.

Therefore, it is fundamental to revise, analyze and complete the existing information about mineral content that are found in food chemical composition tables, databases or nutritional software, in order to create health programs, plan healthy menus and produce quality market products. For the above reasons, the objective of this research is to characterize the proximal analysis and mineral composition of castilla squash (*C. moschata*) seeds, green pea (*P. sativum*) pods, and pinto saltillo green bean pods (*P. vulgaris*), with the aim of identifying a possible use in agroindustrial processes, improving the use of organic residues, and identifying a potential use in Agribusiness 4.0 for the creation of foods that allow to cover the possible deficiencies and demands from the population.

MATERIALS AND METHODS

The proximal content was analyzed of castilla squash seeds with shell, squash seeds without shell, squash seed shell, pea pods (seed and shell), pea seeds, pea pods (sheath), green bean pods (seed and shell), green bean seed, and green bean pods (sheath). As well, the mineral composition was determined in complete samples of castilla squash seeds (*C. moschata*), green pea pods (*P. sativum*) and green bean pods (*P. vulgaris*) (Figure 1), with three replications. The samples were obtained randomly from the municipality of Zacatón, Salinas de Hidalgo, S.L.P., and from the Ejido de Moras, Mexquitic de Carmona, S.L.P., during the month of July 2021. Subsequently, they were transported directly and without storage period to the laboratory for analysis.

The determinations were carried out at the Water-Soil-Plant Laboratory of the San Luis Potosí Campus of Colegio de Postgraduados (22° 63' 22" N and 101° 71' 25" W) and at Laboratory 2 of the Coordinación Académica Región Altiplano Oeste (CARAO) of Universidad Autónoma de San Luis Potosí (22° 38' 28.5" N and 101° 42' 10.0" W).

Ash

Ash content was determined according to AOAC method 942.05/90 (AOAC, 1990) and was expressed as percentage in weight of dry flour.

$$\%\ ashes = (weight\ of\ calcined\ ash\ sample) / (weight\ of\ sample) \times 100 \quad (1)$$

Figure 1. Pea pods (*Pisum sativum*), pinto saltillo green bean pods (*Phaseolus vulgaris*) and castilla squash seeds (*Cucurbita moschata*).

Raw protein

Raw Protein was determined through the Kjeldalh method according to technique 955.04/09 (AOAC, 1990), which determines the nitrogen concentration present in the sample to then be transformed into protein through a factor.

Total carbohydrates

Total carbohydrate content was determined using the method based on AOAC 974.06 (AOAC, 1975).

Total fat

The fat content was determined using the 920.39C procedure described by the AOAC (AOAC, 1990), by means of the Soxhlet technique in a ST243 Soxtec TM Extraction Unit.

Crude fiber

The determination of fiber content was carried out on previously defatted samples, performing acid digestion in the presence of 1.25% H_2SO_4 and alkaline digestion in the presence of 3.52% NaOH, using the AOAC method Weende 962.09/90 (AOAC, 1990).

Open acid digestion

The procedure used is based on that described by Jakubowska *et al.* (2006) with some modifications. For this purpose, 0.5 g of sample was weighed on an analytical scale (OhausAdventurer, model H-5276) and transferred into a flat Teflon tube of 50 mL capacity. Subsequently, 25 ng mL^{-1} of Iridium and Indium were added to each sample as an internal standard to perform the method recovery. In addition, 10 mL of ultrapure concentrated HNO_3 were added and stored at room temperature for 12 h.

The samples with the HNO_3 were placed on a heating plate (NJBZH brand, model BZH29) and heated to conduct evaporation (hot acid digestion), without taking it to dryness; that is, approximately when the tubes had 1 mL of the concentrated sample, 10 mL of concentrated H_2O_2 were added drop by drop in order to destroy all the organic matter present in the sample (this process is also called total mineralization of the sample). Finally, the samples were calibrated to 25 mL in class A volumetric flasks with batch certificate.

Mineral analysis in ICP-MS

For the determination of the 63 mineral elements, the inductively coupled plasma mass spectrometry (ICP-MS) procedure was used with the iCAP™ RQ equipment, in KED mode and with a collision cell.

Statistical analysis

Data analysis was performed in r-project® (4.1.1) software under the RStudio® (2021.09.0) interface, both of free distribution, for which an analysis of variance was performed under a completely randomized design and a means comparison by Tukey's method (α=0.05).

RESULTS AND DISCUSSION

Proximal composition

According to the results of the proximal analysis (dry basis), a significant difference ($p<0.001$) was found in the five determinations. Pea presented the lowest fat content in the pod shell (12300 ppm), the highest protein (320900 ppm) and ash (32400 ppm) content in the seed and the highest total carbohydrate content in the complete pod (shell plus seed) (456000 ppm). Squash had the highest fat content (495000 ppm) and the lowest ash content (10000 ppm) in the shelled seed, while the shell of this species had the highest crude fiber content (711500 ppm) and the lowest total carbohydrate content (61100 ppm) (Table 1).

The values obtained for the proximal content of squash seeds, green bean pods and pea seeds were different from those found in the literature, in several cases due to factors such as determination techniques, types of solvents/reagents, geographical area, environmental factors where they are grown, among other aspects. Rössel-Kipping *et al.* (2018) found lower values from those of this study except for ash content with 6919.99 ppm, crude fiber with 45630 ppm, fat of 283240 ppm, protein of 1290 ppm and carbohydrates with 211190 ppm in castilla squash seeds (*C. moschata*). Similarly, the fat content found in this study (417000 ppm) is higher than that found in castilla squash seeds (*C. moschata*) with 331500 to 346000 ppm (Martinez *et al.*, 2011).

On the other hand, in the case of the whole green bean pod, Fernández-Valenciano and Sánchez-Chávez (2017) obtained carbohydrate values of 346700 ppm and protein 185100 ppm, similar to those of this study with values of 185100 ppm and 377500 ppm, respectively. However, the ash (82400 ppm), fat (13000 ppm) and fiber (7900 ppm) contents are different from those found in this study. As for *Pisum sativum* L., the values found in this study for pea pods (seed and shell), pea seed and pods (sheath) were between 182100 and 320900 ppm of protein, between 12300 and 17200 ppm of fat, and between 12900 and 32400 ppm of ash, values that are in the range of those reported in the literature. Mateos-Aparicio *et al.* (2010) reported 108000 ppm of protein, 13000 ppm fat and 66000 ppm ash. Similarly, Millar *et al.* (2019) report values of 215000 ppm protein, 12800 ppm fat and

Table 1. Mean and standard deviation of proximal concentration of squash seeds, green bean pods, pea pods and their byproducts.

Evaluated product	Protein (mg kg^{-1})	Grasa (mg kg^{-1})	Total carbohydrates totales (mg kg^{-1})	Crude fiber (mg kg^{-1})	Ash (mg kg^{-1})
Pea pod	182100±0.03h*	12300±0.02i	423000±0.37b	307000±0.28b	12900±0.06g
Pea seed	320900±0.03a	16000±0.09g	295800±0.18f	282000±0.05d	32400±0.05a
Pea mix	230600±0.02e	17200±0.02f	456000±0.01a	220800±0.01e	20100±0.04e
green bean pod	183000±0.02g	14000±0.01h	393100±0.02d	290800±0.01c	22400±0.04d
green bean seed	293000±0.02b	23000±0.02d	403900±0.02c	205600±0.03f	16300±0.03f
green bean mix	185100±0.04f	25400±0.03c	377500±0.02e	208000±0.01f	32000±0.03ab
pumpkin seed mix	291500±0.04c	417000±0.05b	66700±0.09h	134000±0.03g	31000±0.05b
shelled pumpkin seed	263000±0.03d	495000±0.03a	149700±0.03g	25800±0.03h	10000±0.05h
Pumpkin seed shell	90800±0.02i	19700±0.02e	61100±0.02i	711500±0.09a	28000±0.04c

*values with the same letter within columns show no significant difference, Tukey ($\alpha=0.05$; $n=3$).

27600 ppm ash for green peas. Rempel *et al.* (2019) studied pea flour and obtained protein values from 420000 ppm to 500000 ppm, fat concentrations from 13300 ppm to 15700 ppm, and ash from 23400 ppm to 25600 ppm.

Meanwhile, Dahl *et al.* (2012) report protein values between 212000 ppm and 329000 ppm, fat 12000 ppm to 24000 ppm, and ash 23000 ppm to 34000 ppm for peas.

Mineral composition

According to the analysis of variance, the content of micronutrients (minerals and trace elements) of the seeds of castilla squash, pea pods and green bean showed significant difference ($p<0.001$). The dietary requirements of minerals in humans vary from a few micrograms per day to one gram per day and are essential for the proper functioning of the heart, the brain, as well as for blood clotting, nerve function, structural reconstruction of body tissues and bones, and are also involved in most metabolic processes, enzymatic reactions and muscle contraction (Ciudad, 2014). It is therefore important to supply these mineral nutrients in the diet. The essential micronutrients for human beings are divided into two classes: minerals that must be supplied in large amounts in the diet (100 mg or more) and essential trace elements that are necessary in only a few mg per day (Daza, 2001).

In relation to the concentrations of minerals in the castilla squash seeds, pea and green bean pods, the green bean pods had a higher content of sodium (139.0584 ppm) and potassium (15626.9991 ppm). On the other hand, squash seeds presented higher concentrations of magnesium (3183.2744 ppm) and phosphorus (9723.1968 ppm), while pea pods presented higher calcium content of 377.4985 ppm (Table 2).

Karanja *et al.* (2013) report the presence of potassium (1240-3350 ppm) in squash seeds which are like those found in this study. Similarly, Hussain *et al.* (2021) found potassium (3877 ppm) and calcium (56.7 ppm) values in squash seeds (*Cucurbita maxima*) similar to those of this research. Chí-Sánchez *et al.* (2020) studied six whole seed variants of squash (*Cucurbita moschata*) and found magnesium dry weight concentrations of between 2960 ppm and 24140 ppm, similar to those in this study which were 3183.27 ppm, in the case of phosphorus concentrations between 1750 ppm and 4370 ppm, potassium from 172100 ppm to 715600 ppm, and calcium from 41000 ppm to 103500 ppm different from those of this study, which were 9723.19 ppm of phosphorus, 3380.09 ppm of potassium and 50.22

Table 2. Mean and standard deviation of mineral concentrations of squash seeds, green bean pods and pea pods.

Evaluated product (mg kg^{-1})	Pumpkin seed	Pea pod	Green bean pods
Calcium	50.22±0.02c*	377.49±0.02a	348.77±0.01b
Potassium	3380.09±0.36c	7349.23±0.03b	15626.99±0.01a
Magnesium	3183.27±0.36a	1719.56±0.02c	1722.16±0.01b
Sodium	116.56±0.03c	130.24±0.02b	139.05±0.02a
Phosphorus	9723.19±0.21a	5438.18±0.02c	6068.44±0.01b

*values with the same letter within rows do not show significant difference, Tukey ($\alpha=0.05$; $n=3$).

ppm of calcium. On the other hand, Fernández Valenciano and Sánchez Chávez (2017) obtained different values for ejojo bean pods of 0.18 ppm, 1.42 ppm, 0.33 ppm and 0.53 ppm of phosphorus, potassium, magnesium and calcium, respectively.

Meanwhile Mateos-Aparicio *et al.* (2010) studied pea pods (*Pisum sativum*) and found magnesium values of 2100 ppm, similar to those of this study (1719.56 ppm), in the case of potassium (10300 ppm) and calcium (7700 ppm) values were different, since those of this research were 7349.23 ppm potassium and 377.49 ppm calcium. Likewise, Millar *et al.* (2019) reported values of 1032 ppm magnesium, 11353 ppm calcium, and 10439.10 ppm potassium in green peas. The difference of the values of this study from those of the literature may be due to several factors that include the variety of squash, the techniques, equipment and solvents used for the determination of the content of these minerals, the climatic and cultivation conditions, as well as the post-harvest storage.

Essential trace elements

Bean pods showed higher contents of molybdenum (4.83011 ppm), iron (100.30591 ppm) and cobalt (0.13818 ppm). On the other hand, squash seeds showed higher concentrations of chromium (3.26827 ppm), manganese (31.832 ppm), copper (6.34767 ppm), zinc (35.43907 ppm) and selenium (0.01465 ppm), compared to pea and green bean pods. Meanwhile, pea pods presented molybdenum (1.42652 ppm), manganese (25.06485 ppm), copper (5.25487 ppm) and selenium (0.00763 ppm), with lower concentrations than squash seeds and green bean pods (Table 3).

The highest concentration in the 3 products evaluated was iron, and it was detected in the highest concentration in the bean pod (100.30591 ppm), followed by high concentrations of manganese, with the squash seed having the highest content of 31.83205 ppm. The concentrations reported by some authors are different from those of this research, but in the studies they report that there was significant difference in the content of essential trace elements between the species evaluated; this indicates the morphological variability present between variants of each species and the potential they have as sources of essential trace elements (Darrudi *et al.*, 2018). Zinc concentrations in this study were 35.43 ppm, similar

Table 3. Mean and standard deviation of the concentration of essential trace elements in squash seeds, green bean pods and pea pods.

Evaluated product (mg kg^{-1})	Pumpkin seed	Pea pod	Green bean pod
Cobalt	0.09±0.02c*	0.098±0.02b	0.13±0.02a
Chromium	3.26±0.02a	2.78±0.01c	3.05±0.01b
Copper	6.34±0.03a	5.25±0.02c	5.42±0.01b
Iron	86.70±0.02c	94.25±0.03b	100.30±0.01a
Manganese	31.83±0.02a	25.06±0.02c	31.02±0.01b
Molybdenum	1.70±0.03b	1.42±0.02c	4.830±0.01a
Selenium	0.01±0.02a	0.01±0.02c	0.01±0.02b
Zinc	35.43±0.02a	28.86±0.8b	19.46±0.02c

*values with the same letter within rows do not show significant difference, Tukey (α=0.05; n=3).

to those reported in other studies ranging from 3.1 to 65.54 ppm (Chí-Sánchez *et al.*, 2020). However, iron, manganese and copper concentrations in whole squash seeds were different from those reported by Chí-Sánchez *et al.*, (2020) with concentrations ranging from 0.93 to 13.75, 0.93 to 4.38 ppm and 0.59 to 3.3 ppm, respectively. Similarly Hussain *et al.* (2021) reported different concentrations of iron (61.6 ppm) and zinc (152.1 ppm) in squash seeds (*Cucurbita maxima*). In the case of green bean and peas pods, the values found are similar with the research by Fernandez Valenciano and Sanchez Chavez (2017) for green bean pod where values of 5.61 ppm copper, 25.21 ppm manganese, 73.45 ppm iron and 19.28 ppm zinc were found, and with those reported by Millar *et al.* (2019) of zinc (38.80 ppm) and iron (33.10 ppm) in green peas.

Toxic trace elements

It is necessary to know the amount of minerals found in foods for human consumption, especially minerals whose atomic weight is between 63.55 and 200.59, and depending on the concentration in which their ions and compounds are considered toxic to humans and the environment. Some minerals considered in this category are: Thallium, Aluminum, Antimony, Barium, Fluorine, Arsenic, Cadmium, Cobalt, Chromium, Copper, Mercury, Nickel, Lead, Tin and Zinc (Londoño-Franco *et al.*, 2016).

Table 4 shows the results of the data obtained for the main toxic trace elements in both squash seeds and pea and green bean pods; squash is the product studied with the highest amount of toxic trace elements, the main ones being antimony (0.11372 ppm), arsenic (0.04697 ppm), mercury (0.0233 ppm), lead-208 (0.08066 ppm) and tin (90.0679 ppm).

The highest concentration of toxic trace elements was found in the green bean pods, while the lowest concentration was found in squash seeds. According to Berdonces (1996), data from the table of estimated daily intake of metals and trace elements and toxicity, the results do not exceed the maximum allowed daily intake limits for cadmium, arsenic, thallium, antimony, and lead; that is, up to 500 g of squash seeds can be consumed and do not exceed the allowed daily limit of mercury. There is little information on the toxic trace elements in whole squash seeds, pea pods and whole green beans. However, Fernández Valenciano and Sánchez Chávez (2017) obtained similar values of nickel (4.00 ppm) in ejojo bean pods to what was found in this study (3.70 ppm).

In general, heavy metal contamination is commonly associated with municipal and industrial discharges (inorganic waste, solid discharges of hazardous waste and domestic and industrial garbage) which go directly into water bodies; however, in this study the main factor that may explain the high concentrations of aluminum and tin is perhaps due to organic contamination and the use of agrichemicals applied before and during the production of squash, pea and green bean. According to Berdonces (1996), aluminum and tin exceeded the allowed limits in the three study products evaluated, which are different in each country.

Similarly, there were no concentrations of heavy metals such as europium, terbium, holmium, thulium, ytterbium, lutetium and rhenium in these seeds and pods, and low concentrations of gold, hafnium, erbium, dysprosium, gadolinium, samarium and praseodymium were observed.

Table 4. Mean and standard deviation of the concentration of toxic trace elements in squash seeds, green bean pods and pea pods.

Evaluated product (mg kg^{-1})	Pumpkin seed	Pea pod	Green bean pod
Aluminum	25.16±0.00c*	51.38±0.02a	45.94±0.01b
Silver	0.10±0.02a	0.06±0.01c	0.07±0.02b
Arsenic	0.04±0.02a	0.02±0.02c	0.04±0.02b
Barium	0.65±0.02c	7.21±0.02a	4.61±0.01b
Beryllium	0.01±0.01c	0.01±0.03a	0.01±0.01b
Cadmium	0.02±0.02b	0.01±0.01c	0.02±0.00a
Cesium	0.01±0.02c	0.07±0.02a	0.02±0.02b
Galium	0.01±0.02c	0.01±0.01b	0.01±0.01a
Mercury	0.02±0.01a	0.02±0.01b	0.01±0.02c
Lithium	0.15±0.08c	0.24±0.01b	0.30±0.02a
Nickel	2.41±0.03b	2.04±0.02c	3.70±0.02a
Lead 206	0.05±0.02a	0.01±0.02c	0.04±0.01b
Lead 207	0.05±0.01a	0.03±0.01c	0.05±0.01b
Lead 208	0.08±0.03a	0.07±0.01b	0.05±0.03c
Rubidium	3.39±0.02c	22.26±0.02a	16.28±0.01b
Antimony	0.11±0.03a	0.09±0.01c	0.10±0.01b
Tin	90.06±0.02a	40.50±0.03c	65.77±0.01b
Strontium	4.13±0.03c	26.10±0.02a	19.27±0.02b
Titanium	1.62±0.01c	12.06±0.00a	8.77±0.01b
Thallium	NP	0.02±0.01	NP
Uranium	0.01±0.00b	0.01±0.01c	0.01±0.02a
Vanadium	0.06±0.03c	0.09±0.02a	0.07±0.01b
Boron	2.51±0.00c	4.59±0.04b	4.87±0.02a
Bismuth	0.039±0.03c	0.05±0.01a	0.04±0.01b
Thorium	0.01±0.03b	0.01±0.02a	0.01±0.02a
Scandium	0.01±0.04b	0.01±0.01a	0.01±0.01a
Germanium	0.01±0.01c	0.01±0.02b	0.01±0.01a
Yttrium	0.01±0.02c	0.01±0.01b	0.02±0.01a
Zirconium	0.10±0.03b	0.07±0.02c	0.11±0.01a
Niobium	0.03±0.03a	0.02±0.02c	0.02±0.01b
Tellurium	0.01±0.02a	0.01±0.02c	0.01±0.01b
Lanthanum	0.01±0.02c	0.01±0.02b	0.01±0.01a
Cerium	0.02±0.03c	0.02±0.02b	0.03±0.01a
Praseodymium	0.01±0.02c	0.01±0.02b	0.01±0.01a
Neodymium	0.01±0.02c	0.01±0.00b	0.01±0.01a
Samarium	0.01±0.02c	0.01±0.02b	0.01±0.01a
Europium	NP	NP	NP
Gadolinium	0.01±0.02c	0.01±0.01b	0.01±0.00a
Terbium	NP	NP	NP
Dysprosium	0.01±0.02c	0.01±0.01b	0.01±0.02a
Holmium	NP	NP	NP
Erbium	0.01±0.02c	0.01±0.02a	0.01±0.02b
Thulium	NP	NP	NP
Ytterbium	NP	NP	NP
Lutetium	NP	NP	NP
Hafnium	0.01±0.01a	0.01±0.02b	0.01±0.01b
Tantalum	0.01±0.03a	0.01±0.01b	0.01±0.01c
Tungsten	0.01±0.01b	0.01±0.01a	0.01±0.01c
Rhenium	NP	NP	NP
Gold	0.01±0.01	NP	NP

*values with the same letter within the rows do not present significant difference, Tukey (α=0.05; n=3), where: NP=did not present.

To achieve optimal use of the products evaluated, the combined use of squash seeds, pea pods and green beans is recommended to help combat malnutrition with natural foods, to design diets for recovery of chronic patients and to prevent deficiency states in the general population (Cáceres and Cruz, 2019). These results also contribute to complete information on the chemical composition of foods or to generate databases such as the Swiss table (Switzerland, 2021).

CONCLUSIONS

According to the results, the shells of squash seeds, pea pods and green beans have a high composition of fiber, carbohydrates, protein and minerals, so they can be considered as an important source of essential nutrients and could be an alternative in the future to complement the commercial flours used also as natural or synthetic foods by the population.

The minerals with the highest concentrations in shelled squash seeds, complete pea and green bean pods were potassium, phosphorus and magnesium and the minerals with the lowest concentrations were erbium and dysprosium, while the concentrations of toxic minerals are very low and no concentrations of rhenium, lutetium, ytterbium, thulium, holmium, terbium and europium were found. For this reason, whole seeds and pods are an alternative for the sustainable development of food technology, health and nutrition, without causing environmental contamination. However, information is scarce to generate technology products, food and non-food; therefore, research is needed to use information optimally, rationally and effectively in Agribusiness 4.0 and for the consumer without harming the environment.

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