

Genetic variability of nanche in Tabasco, Mexico, determined with RAPDs

Variabilidad genética del nanche en Tabasco, México, determinada con RAPDs

Martínez ME¹, JM Leshner G², G Castañón N², E de la Cruz L¹, C Zapata H²

Abstract. Nanche (*Byrsonima crassifolia* (L.) H.B.K.) grows in the southeastern region of the Mexican republic under both wild and semi-cultivated forms. Despite the importance of the fruit of this tree, only one paper at the molecular level has been published in Mexico, and possibly in America, to date. For this reason, the purpose of this research was to study the genetic variability of nanche trees that grow under both wild and semi-cultivated conditions in the state of Tabasco, Mexico. Molecular determinations were carried out in the Laboratorio de Genómica of the División Académica de Ciencias Biológicas (DACBiológicas), Universidad Juárez Autónoma de Tabasco (UJAT), using the RAPD (Random Amplified Polymorphic DNA) technique. Of the six evaluated oligonucleotides, only four amplified 10.3 bands each one on average, and of these, three presented polymorphism (2,8 polymorphic bands). Thus, we consider convenient to use other primers or molecular markers in future studies on nanches. Dice's analysis detected six groups, and the cophenetic correlation coefficient was $r=0.82$, indicating a good formation of groups of the evaluated material. The average genetic diversity (H_i) presented a moderate value (0.12). According to the analysis of molecular variance (AMOVA), genetic diversity was greater within (85.20%) than among populations (14.80%), and the fixation index (F_{st}) was 0.148. These results indicate that the RAPD technique was useful for identify the variation of the *Byrsonima crassifolia* (L.) H.B.K., and this is very valuable for the in situ maintenance of a nanche germplasm bank.

Keywords: Genetic resources; Molecular markers; *Byrsonima crassifolia* (L.) H.B.K.; Genetic diversity; AMOVA; Chunga; Chi.

Resumen. En el sureste de la república Mexicana, el nanche (*Byrsonima crassifolia* (L.) H.B.K.) crece en forma silvestre o semi-cultivada. No obstante la importancia que tiene el fruto de este árbol, a la fecha solo se reporta un trabajo desarrollado a nivel molecular en México, y posiblemente en América. El propósito de esta investigación fue estudiar la variabilidad genética de árboles de nanche, que crecen en forma silvestre y semi-cultivada en el estado de Tabasco, México. La determinación molecular se realizó en el Laboratorio de Genómica de la División Académica de Ciencias Biológicas (DAC Biológicas), Universidad Juárez Autónoma de Tabasco (UJAT), usando la técnica de RAPDs (Polimorfismo Amplificado Aleatoriamente de ADN). De los seis oligonucleótidos evaluados, solo cuatro amplificaron 10,3 bandas cada uno en promedio, y de éstos, tres mostraron polimorfismo (2,8 bandas polimórficas). Esto sugiere la conveniencia de probar otros iniciadores o marcadores moleculares en futuros trabajos en nanche. El análisis de Dice detectó seis grupos, y el valor del coeficiente de correlación cofenética fue de $r=0.82$, lo que indica una buena agrupación del material evaluado. La diversidad genética promedio (H_i) presentó un valor moderado (0,12). De acuerdo con los resultados del análisis molecular de varianza (AMOVA), la diversidad genética fue mucho más grande dentro (85,20%) que entre poblaciones (14,80%), y el índice de fijación (F_{st}) fue de 0,148. Estos resultados hacen suponer que la técnica de RAPDs es útil para identificar la variación de *Byrsonima crassifolia* (L.) H.B.K., lo cual es muy valioso para el mantenimiento de un banco de germoplasma *in situ*.

Palabras clave: Recursos genéticos; Marcadores moleculares; *Byrsonima crassifolia* (L.) H.B.K.; Diversidad genética; Nanche; Chunga; Chi.

¹ División Académica de Ciencias Agropecuarias, Universidad Juárez Autónoma de Tabasco. Ra. La Huasteca 2ª Sección. Kilómetro 25,0, Carretera Villahermosa-Teapa.

² División Académica de Ciencias Biológicas, Universidad Juárez Autónoma de Tabasco. Carretera Villahermosa-Cárdenas, kilómetro 0,5, Entronque Bosques de Saloya, Villahermosa, Tabasco, México. C.P 86150, Tel. y Fax 055-993-3-54-43-08.

Address Correspondence to: Guillermo Castañón Nájera, e-mail: guillermo_corazon_valiente@hotmail.com, guillermo_rasputin956@hotmail.com

Recibido / Received 9.II.2012. Aceptado / Accepted 26.VII.2012.

INTRODUCTION

Mexico harbors a great genetic diversity of autochthonous plants, like the nanche. Nanche (*Byrsonima crassifolia* (L.) H.B.K.) is an heliofito tree, hermaphrodite flowers, pentamerous calyx with five triangular green sepals, oval, and each one with two glands at the base, corolla with five yellow petals with orange taken daily with a hairy claw at the base; stamens ten in number, anthers oblong, basifixed, bilocular, ovary glabrous, ovoid-oblong and with three styles ending in three stigmas acute. It is a native tree to Mesoamerica (Humbolt & Bonpland, 1822). Nava & Uscanga (1978) indicated that the nanche presents great phenotypic variability due to natural cross-pollination and seed propagation, manifested in the existence of a large variety of sizes, color flavor, aroma and fruit shape. In Mexico, nanches are distributed throughout the tropical region, growing from the south of Tamaulipas and east of San Luis Potosí to the Yucatán peninsula, where it is known as Nanche, Nance, Nanchi or Nanantze. Along the Pacific watershed, it is distributed from the south of Sinaloa to Chiapas states (Humbolt & Bonpland, 1822).

Nanche fruits and plants are very important to the rural population as they are used (1) for medical and feeding purposes, (2) as a source of vitamin C, (3) have a high nutritional value, (4) as wood, decorations and fuel, (5) to extract dye, and (6) for preparation of alcoholic beverages, ice cream and salads (Humbolt & Bonpland, 1822; Castillo-Campos et al., 2002; Medina-Torres et al., 2004; Martínez-Moreno et al., 2006).

In Central America and the Caribbean islands, the nanche also exists. In South America, nanche is found in Perú, Bolivia, Paraguay and Brazil. In these countries, if nanche is grown on fertile soils and good crop management is used, a mature plant can produce up to 20 kg of fruit per year. In soils with low fertility, production can be as low as to 7 kg/plant (Anónimo, 2012). The plant can adapt to a wide range of climatic conditions and can grow and develop in warm, semi-warm and/or temperate latitudes, generally on degraded soils (Vázquez-Yanes et al., 1999; FAO, 2006). Despite the importance of nanche, only one molecular study on wild and cultivated nanche plants has been reported in Mexico (Raya-Pérez et al., 2010).

One way to determine with certainty the genetic structure of a population is using molecular markers. These constitute a useful tool to determine the variability that may exist among varieties, and within wild and cultivated populations of a species of interest (Mitton, 1994; Rodríguez & Arencibia, 2002). The main advantages of considering molecular characteristics are that these are universal and are not affected by the environment. RAPD (Random Amplified Polymorphic DNA) are markers that randomly amplify DNA segments of a great variety of species. The RAPDs technique was developed by Williams et al. (1990) and, notwithstanding its limitations, has proved to be an important tool in the evaluation of the genetic constitution of different plant species. RAPDs have been applied in studies on woody plants such as Yacón (*Smilanthus sonchifolius*: Mansilla et al., 2006), pine (*Pinus patula*:

Table 1. Origin, geographical location and morphologic identification of the nanche (*Byrsonima crassifolia* (L.) H.B.K.) trees sampled for molecular characterization.

Tabla 1. Origen, ubicación geográfica e identificación morfológica de los árboles de nanche (*Byrsonima crassifolia* (L.) H.B.K.) muestreados para la caracterización molecular.

Tree number	Municipality	Geographical location	Fruit Color
1	Centro	17° 59' 54" N; 92° 55' 42,5" W	Yellow
2	Centro	17° 59' 57,5" N; 92° 55' 46,7" W	Yellow
3	Centro	17° 58' 38,2" N; 92° 56' 37,9" W	Yellow
4	Centro	17° 58' 38,3" N; 92° 56' 37,0" W	Yellow
5	Centro	17° 58' 41,2" N; 92° 56' 33,2" W	Green
6	Centro	17° 58' 41,6" N; 92° 56' 33,0" W	Green
7	Centro	17° 58' 42" N; 92° 56' 32,9" W	Green
8	Centro	17° 58' 25,8" N; 92° 56' 26,2" W	Yellow
9	Centro	17° 58' 25,6" N; 92° 56' 26,5" W	Green
10	Huimanguillo	17° 39' 00,5" N; 92° 23' 55,7" W	Red
11	Huimanguillo	17° 38' 56,5" N; 93° 23' 42,1" W	Green
12	Huimanguillo	17° 38' 56,6" N; 93° 23' 46,9" W	Yellow
13	Huimanguillo	17° 38' 56,5" N; 93° 23' 51" W	Yellow
14	Huimanguillo	17° 38' 56,5" N; 93° 23' 51" W	Yellow
15	Huimanguillo	17° 58' 50,2" N; 92° 23' 47,6" W	Red

Luna-Rodríguez et al., 2005) and chago, miso or mauka (*Mirabilis expansa*: Chia et al., 2006).

The purpose of this research was to molecularly characterize the genetic variability of nanche trees found in two municipalities in the state of Tabasco, Mexico.

MATERIALS AND METHODS

Plant material and sampling area. Plant material was collected (9 trees) in the city of Villahermosa (Centro municipality) and in several ranches in the municipality of Huimanguillo (6 trees), both in the state of Tabasco, Mexico. The material was collected during September to October 2008, from 9 to 12 young leaves, unblemished by diseases and insects. They were collected from each tree found at the sampling sites. After cutting, each leaf was collected before touching the ground. Each sampled tree was graded visually for flower color, and fruit color and size during sample collection. Table 1 presents the geographical description and location of the trees that were sampled at each site.

DNA extraction and RAPD analyses. DNAg was extracted following the CTAB method (Cetyl Trimethyl Ammonium Bromide) developed by Doyle & Doyle (1990) and modified by Bekesiova et al. (1999). The PCR (Polymerase Chain Reaction) took place in 20 μ L of final volume reaction. Each reaction contained per sample: 10 μ L of QTM Supermix (BIORAD), 8 μ L of sterile water, 1 μ L primer and 1 μ L with 20 ng of DNAg. The DNAg was amplified with the oligonucleotides (Probiotek brand) OPB-2 (5'TGATCCCTGG3'), OPB-10 (5'CT-GCTGGGAC3'), OPB-18 (5'CCACAGCAGT3'), OPC-5 (5'GATGACCGCC3'), OPE-4 (5'GTGACATGCC3') and SC10-37 (5'GCCAATCCTG 3'). Once the reaction was prepared, the DNAg samples were placed in the PCR MyCycler thermocycler (My CyclerTM, BIORAD). The initial denaturalization temperature was 94 °C for 4 min, followed by the PCR final point program that consists of 33 cycles at 94 °C for 1 min, 58 °C for 1:30 min and 72 °C for 2 min, plus 1 additional cycle for the final extension at 72 °C for 5 min, to end with 1 cycle at 4 °C (infinite). Once the PCR was complete, the amplified products were stored at -4 °C. Thereafter, the PCR products were separated in 1.5% agarose gel with a TAE 1X buffer solution, and 80 volts were applied during 2 hours. The DNA bands were visualized with ethidium bromide, and the molecular weight (MW) of each band was compared with the MW of the Hyperlader I marker (Bioline brand). After this, a photograph of each gel (where there were the PCR products amplified bands) was taken in an ultraviolet camera obscura [Uvisave GAS200 (UVITEC)], and these were analyzed with the UVigeltec Version 12.0 software (UVITEC).

Data analyses. A binary matrix for presence (1) and absence (0) was constructed with the total bands that were obtained (polymorphic and no polymorphic bands), and only color fruit

was incorporated, transformed this to a binary scale too (IPGRI, 2004). Genetic distances were calculated with the Dice's method (Nei & Li, 1979), and estimated as
$$\frac{2a}{(2a + b+c)}$$
.

The cluster analysis for the comparison of genetic relationships among the sampled trees was carried out according to Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm using Free tree and Tree view software (Page, 1996). The re-sampling (Bootstrap) applied to the binary data matrix was of 1000. The criterion for cluster formation where the line starts (0.1), and the percentage of times (numbers in the nodes) that presented the same group (Hampl et al., 2001). The cophenetic correlation coefficient was estimated using NTSYS-PC software (Rohlf, 1997). The polymorphism levels found with each oligonucleotide was determined following Infante et al. (2006) and Gil et al. (2001). The proportion of polymorphic loci (P) was calculated as
$$P = \frac{NLP}{TL}$$
.

where NLP=number of polymorphic loci, TL=total loci. The diversity of each locus was estimated as $h_j = 1 - p^2 - q^2$, where p_i and q_i are the frequency of the i and j alleles. The average genetic diversity was calculated with the expression

$$H_i = \frac{\sum_i^L h_j}{L}$$

where H_i represents the total of the average genetic diversity,

$\sum_i^L h_j$ is the sum of the genetic diversity of each locus, and L is the number of locus (IPGRI, 2004). Using the GenAlEx 6.4 Software (Peakall & Smouse, 2006), an analysis of molecular variance (AMOVA) was performed to split the total genetic variation within and among populations (Weir, 1996), and the Fixation index (Fst) was estimated too.

RESULTS

RAPDs analysis. Of the six evaluated oligonucleotides, only four primers amplified bands (Table 2) on average varied in size from 400 to 2500 pb. The oligonucleotides OPB-2 and OPB-10 did not present amplification of bands. The primers OPC-5, OPE-4 and SC10-37, presented polymorphic bands, while OPB-18 showed no polymorphism in their bands (Table 2).

The dendrogram (Fig. 1) obtained with the similarity coefficient of Dice's and UPGMA allowed the formation of six groups. In general, the trees were grouped by their origin or locality (except group five, where a tree from Villahermosa clustered with two of Huimanguillo). The fruit color did not allow a clear definition of clusters, and the exception was cluster six (green fruit). The other clusters gathered trees with different fruit color; the 14th tree was a single cluster. This is corroborated by the values estimated by the Bootstrap (Fig. 1). Of the six clusters, the lowest value (58.0%) as that of the group with

trees 1 and 6, which were sampled in the city of Villahermosa. The highest values (79.0% and 89.0%) were those of the groups with trees 2, 3, 4 and 5, and 7 and 9 also of Villahermosa. The trees in the ranches in the municipality of Huimanguillo also formed groups, such as that with trees 12, 13 and 15, with a value of 69.0%, and other group was formed with two trees of Huimanguillo and one of Villahermosa (10, 11 and 8) which presented a low percentage (22%). Finally, tree 14 formed a sin-

Table 2. Comparison of the polymorphism shown by each of the RAPDs oligonucleotides used on nanche (*Brysonima crassifolia* (L.) H.B.K.).

Tabla 2. Comparación del polimorfismo mostrado por cada uno de los oligonucleótidos RAPDs usados en nanche (*Brysonima crassifolia* (L.) H.B.K.).

Oligonucleotide	Number of Bands			Polymorphism (%)
	Total	Monomorphic	Polymorphic	
OPB-18	9	9	0	0.00
OPC-5	14	8	7	50.00
OPE-4	11	9	2	18.18
SC10-37	7	6	2	33.33
Band Total	41	32	11	25.38

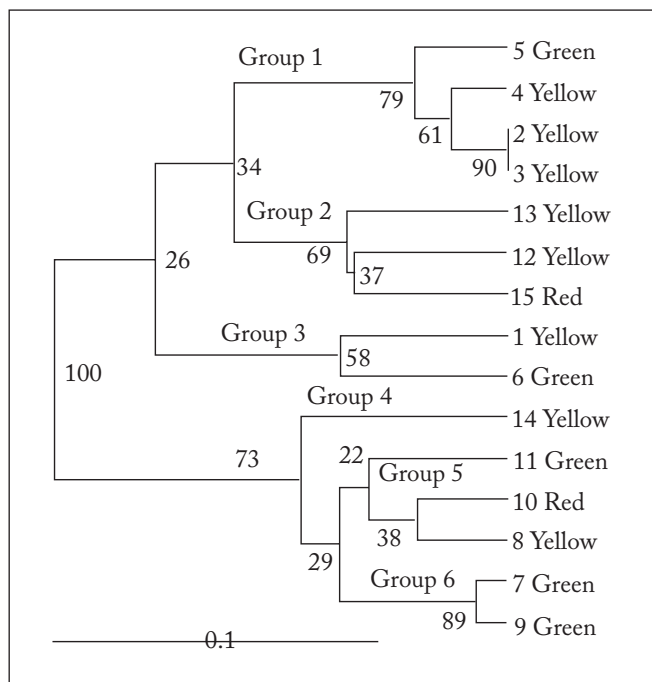


Fig. 1. Dendrogram of nanche (*Brysonima crassifolia* (L.) H.B.K.) trees, UPGMA, (Dice) method with RAPDs type markers.

Fig. 1. Dendrograma en árboles de nanche (*Brysonima crassifolia* (L.) H.B.K.) por el método UPGMA (Dice) con marcadores tipo RAPDs.

gle cluster and the color was not properly defined in the cluster (note), except for the cluster tree six (green fruit). The other clusters gathered trees with a different fruit color (Hapl et al., 2001). A comparison between the matrix obtained following Dice's and the cophenetic matrix provided a value of $r=0.82$. This would indicate that the formation of groups of the sampled individuals in this study was good (Rohlf, 1997). The proportion of polymorphic loci was 25.37%. The genetic diversity within a locus (h_j) varied from 0.38 to 0.50 (data not shown), and the average genetic diversity (H_j) was 0.12.

Population genotypic diversity. Genetic variation was greatest within that among populations, as indicated by the sum of squares values calculated by AMOVA (Table 3). Of the total genetic diversity, 14.80% was attributable to differences among populations and 85.20% was to differences within populations. The fixation index (F_{st}) was 0.148.

Table 3. Molecular analysis of variance (AMOVA) using RAPDs in nanche (*Brysonima crassifolia* (L.) H.B.K.).

Tabla 3. Análisis molecular de varianza (AMOVA) usando RAPDs en nanche (*Brysonima crassifolia* (L.) H.B.K.).

Source of variation	d.f.	SSD	CV	% of Variance	P
Among populations	1	14.99	0.074	14.80	<0.0001
Within populations	13	177.38	0.427	85.00	<0.0001
Total	14	192.38	0.502		

d.f., degrees of freedom; SSD, sum of square deviations, CV, variance component estimates; p, probability of obtaining a larger component estimate.

DISCUSSION

The fact that the primers OPB-2 and OPB-10 did not present amplification of bands may respond to the lack of homologous regions in the genome of the species under study. The bands found in this study are less than those reported by Góes et al. (2009) for Barbados cherry, a tree of the family Malpighiaceae to which nanche belongs. On the other hand, Gulhan et al. (2004) evaluated 12 RAPDs different primers in Turkish sesame. Of these, the primers K2, K1, K8 and K11 accounted for 16, 9, 8 and 8 polymorphic bands. The number of polymorphic fragments was 61 (78.0%) with a mean of 8.7 per primer, values greater than those found in our research. However, the results of Gulhan et al. (2004) regarding the number of bands are similar to those shown by Noeparvar et al. (2008) in alfalfa (*Medicago sativa*). This is proof of how different species and used primers respond to the application of molecular markers, Bahrami et al. (2009), for example, tested 100 RAPD primers; the level of polymorphism was 8.6 bands

polymorphic by primer from the 21 primers that detected polymorphism. The low and null polymorphism found in our study (Table 2) indicates that the genetic variability is small in the evaluated material. However, the results may also respond to the low efficiency of the primers to recognize homologous regions in the DNA of the evaluated trees. The results reported in our study agree with those of Spergiorin et al. (2002) for Barbados cherry, possibly because the tress might be closely related or because they were reproduced vegetatively by producers. This also coincides with findings of Raya et al. (2010) who recorded a high degree of relationship between wild and cultivated nanche trees.

The dendrogram obtained with the molecular data presents a certain coincidence with the results reported by Martínez-Moreno et al. (2006). These authors characterized morphometrically the fruits of *B. crassifolia*, and found that the evaluated material formed well defined groups, based on the fruit color. In our research only the group six was formed with trees that have the same color (green).

The results obtained in this study establish that the polymorphism that may be found in the evaluated material depends on the species and the primers that are used (Gulhan et al., 2004). Thus, for example, the value of the average genetic diversity ($H_i=0.12$) obtained here differs markedly from the values (0.343 to 0.381) recorded by Díaz et al. (2001) for pine (*Pinus oocarpa*) populations. However, the F_{st} is from a moderate to a large value according to IPGRI (2004). Also, according to the AMOVA analysis, genetic variation within populations was 85% of the total variation (Table 3).

According to our results, the polymorphism detected in the evaluated material was low. The differences recorded for the oligonucleotides, when polymorphism was detected, indicate that the primers that detected most of the amplifications should be used in future nanche studies. Additionally, other new oligonucleotides and a greater number (for instance, 20 oligonucleotides) should be tested to find a greater number of polymorphic bands. It may also be convenient to use other types of molecular markers such as, for example, the AFLP (Amplified Fragment Length Polymorphism). The formation of tree groups responded in general to their origin and fruit color, which leads to believe that the trees were obtained from the same germoplasmic source. This is corroborated by the low genetic diversity value recorded for the evaluated material.

REFERENCES

- Anónimo (2012). Indano (*Byrsonima crassifolia*). <http://agronomoglobal.blogspot.mx/2010/02/indano-byrsonima-crassifolia.html>. Fecha de consulta 10 de Julio de 2012.
- Bahrami, R.M., M.E. Hassani, A. Mohammadi, Sh. Lessan & S. Ghazi Zade (2009). Evaluation of genetic diversity in *Capsicum* spp. as revealed by RAPD markers. *Acta Horticulturae* 829: 275-278.
- Bekesiova, I., J.P. Nap & L. Mlynarova (1999). Isolation of high quality DNA and RNA from leaves of the carnivorous plant *Drosera rotundifolia*. *Plant Molecular Biology Reporter* 17: 269-277.
- Castillo-Campos, G. & M.E. Medina (2002). Árboles y arbustos de la reserva natural de la Mancha, Veracruz. Instituto de Ecología, A. C. Xalapa, Ver., 144p.
- Chia, W. J.A., C.F. López B., R. Blas S., J. Seminario, R. Mansilla & J.P. Baudoin (2006). Diversidad genética molecular de *Mirabilis expansa* mediante RAPD. *Ecología Aplicada* 5: 81-86.
- Díaz, V., L.M. Muñoz & E. Ferrer (2001). Random amplified polymorphic DNA and amplified fragment length polymorphism assessment of genetic variation in Nicaragua of *Pinus oocarpa*. *Molecular Ecology* 10: 2593-2603.
- Doyle, J.J. & J.L. Doyle (1990). Isolation of plant DNA from fresh tissue. *Focus* 12: 13-15.
- FAO (2006). Fichas técnicas. Productos frescos y procesados. IICA, Costa Rica. http://www.fao.org/inpho_archive/content/documents/vlibrary/AE620s/Pfrescos/NANCE.HTM. (Fecha de consulta 6/12/2011).
- Gil, V.K., Ch. M. González, O. Martínez de la V., J. Simpson & G. Vandemark (2001). Analysis of genetic diversity in *Agave tequilana* var. azul using RAPD's markers. *Euphytica* 119: 335-341.
- Góes, O.M., J. Gonçalves de O, F.A. Gomes, P.M. Gonzaga, V.A. Pio, F.G. Apolinário de S & L.G.E. Machado (2009). Diversidade genética de Acerolierias (*Malpighia emarginata* D.C.), utilizando marcadores RAPD e Características morfoagronômicas. *Revista Brasileira de Fruticultura* 31: 162-170.
- Gulhan, E.A., M. Taskin & K. Turgut (2004). Analysis of genetic diversity in Turkish sesame (*Sesamum indicum* L.) populations using RAPD markers. *Genetic Resources and Crop Evolution* 51: 599-607.
- Hapl, V., A. Pavlíček & J. Flegr (2001). Construction and bootstrap analysis of DNA fingerprinting-based phylogenetic trees with the freeware program Freetree: application to trichomonad parasites. *International Journal of Systemic Ecology and Mycology* 51: 731-735.
- Humbolt, A. Von & A. Bonpland (1822). *Byrsonima crassifolia* (L) Kunth. *Nova Genera et Species Plantarum* 5: 149. [Fecha de consulta 4/11/2011].
- Infante, D., S. Molina, J.R. Demey & E. Gámez (2006). Asexual genetic variability in *Agavaceae* determined with inverse sequence-tagged repeats and Amplification Fragment Length Polymorphism Analysis. *Plant Molecular Biology Reporter* 24: 205-217.
- IPGRI (2004). Análisis de la diversidad genética utilizando datos de marcadores moleculares: Módulo de aprendizaje. Instituto Internacional de Recursos Fitogenéticos (IPGRI), Roma, Italia. [http://www.ipgri.cgiar.org/Training/unit10-1/Mol_Markers_es/PDF/Vol2/VIII.copy.pdf]. Fecha de consulta 12 noviembre de 2011.
- Luna-Rodríguez, M., J. López-Uptón & L. Iglesias- Andreu (2005). Variabilidad morfométrica y molecular (RAPD) en una plantación de *Pinus patula* en Veracruz, México. *Agrociencia* 39: 231-235.
- Mansilla, R.C., C. López, R. Blas, J. Chia & J. Baudoin (2006). Análisis de la variabilidad molecular de una colección peruana de *Smilanthus sonchifolius* (Poepp & Endl) H. Robinson "Yacón". *Ecología aplicada* 5: 75-80.
- Martínez-Moreno, E., T. Corona-Torres, E. Avitia-García, A.M. Castillo-González, T. Terrazas-Salgado & M.T. Colinas-León (2006). Caracterización morfométrica de frutos y semillas de

- nanche (*Byrsonima crassifolia* (L.) H. B. K.). *Revista Chapingo serie Horticultura* 12: 11-17.
- Medina-Torres, R., S. Salazar-García & J.R. Gómez-Aguilar (2004). Fruit quality indices in eight Nance [*Byrsonima crassifolia* (L.) H.B.K.] selections. *HortScience* 39: 1070-1073.
- Mitton, J.B. (1994). Molecular approaches to population biology. *Annual Review of Ecology and Systematic* 25: 45-69.
- Nava, K.G.G. & M.B. Uscanga (1978). Contribución al estudio de nueve selecciones de *Spondias* sp. y 17 selecciones de *Byrsonima crassifolia* L. en dos regiones del estado de Veracruz. In: CONAFRUT. Memoria del Simposium La investigación, el desarrollo experimental y la docencia. CONAFRUT, México, pp. 819-834.
- Nei, M. & W.H. Li (1979). Mathematical model for studying genetic variation in terms of restriction endonucleasas. *Proceedings of the National Academy of Sciences USA* 76: 5269-5273.
- Noeparvar, S., M. Valizadeh, H. Monirifar, H.A. Razban & B. Darbani (2008). Genetic diversity among and within alfalfa populations native to Azerbaijan based on RAPD analysis. *Journal of Biological Research-Thessaloniki* 10: 159-169.
- Page, R.D.M. (1996). Freetree, Treeview: An application to display phylogenetic trees on personal computers. *Computer Applications into Biosciences* 12: 357-358.
- Peakall, R. & P.E. Smouse (2006). GENALEX6.4: genetic analysis in Excel. Population software for teaching and research. *Molecular Ecology Notes* 6: 288-295.
- Raya-Pérez, J.C., C.L. Aguirre-Mancilla, K. Gil-Vega & J. Simpson (2010). La domesticación de plantas en México: Comparación de la forma cultivada y silvestre de *Byrsonima crassifolia* (Malpighiaceae). *Polibotánica* 30: 239-256.
- Rodríguez, M. & A. Arencibia (2002). Principales tipos de marcadores del polimorfismo de los ácidos nucleicos. Técnicas analíticas. In: Marcadores moleculares: Nuevos horizontes en la genética y la selección de las plantas. Editorial Félix Varela, La Habana, pp 13-35.
- Rohlf, F.J. (1997). NTSYS-PC Numerical Taxonomy and Multivariate Analysis System, Versión 2.0. Exeter Software, Setauket., N.Y.
- Spergiorin, S.M.F., R.C. De Fátima, P.M. Ruas & V. Carpentieri-Pípolo (2002). Uso de Marcadores Moleculares na Análise da Variabilidade Genética em Acerola (*Malpighia emarginata* D.C.). *Revista Brasileira de Fruticultura* 24: 15-22.
- Vázquez-Yanes, C., B.I.M. Batis, M.I.S. Alcocer, M.D. Gual & C. Sánchez-Dirzo (1999). Árboles y arbustos potencialmente valiosos para la restauración ecológica y la reforestación. Reporte técnico del Proyecto J084. Comisión Nacional de Biodiversidad. Instituto de Ecología. Universidad Nacional Autónoma de México. pp. 83-84.
- Weir, B (1996). Genetic Data Analysis II. Methods for Discrete Population Genetic Data. Sinauer Associates, Inc. Publishers, Massachusetts. 2nd ed. 445 p.
- Williams, J.G., A.R. Kubelik, K.L. Livak, J.A. Rafalski & S.V. Tingey (1990). DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18: 6531- 6535.