



UNIVERSIDAD NACIONAL DEL SUR

TESIS DE DOCTORADO EN AGRONOMIA

**Variabilidad genética en germoplasma argentino de
manzano silvestre (*Malus spp*)**

Ing. PAULA CECILIA CALVO

BAHÍA BLANCA

ARGENTINA

2013

Resumen

El manzano (*Malus x domestica* Borkh.) es una de las especies frutícolas más importantes y Argentina es uno de los principales productores de manzanas del hemisferio sur.

Desde su origen, en el centro de Asia, ha experimentado una gran expansión y selección, producto de los objetivos de agricultores y programas de mejoramiento.

Las variedades con predominio a nivel mundial son muy pocas, consecuentemente la especie ha sufrido procesos de erosión genética en las últimas décadas. Con el fin de aumentar la variabilidad genética se ha propuesto incrementar el número de entradas de cultivares primitivos y tipos silvestres en los bancos de germoplasma, asegurando su disponibilidad para ser incorporados en programas de mejoramiento o para su cultivo directo.

El objetivo de este trabajo fue coleccionar y conservar manzanos silvestres en la región cordillerana, determinar la variabilidad genética de los materiales coleccionados mediante marcadores isoenzimáticos y moleculares y evaluar la distribución de la variabilidad genética en las poblaciones.

Se relevaron 68 poblaciones de manzanos silvestres en un área comprendida entre 38° 50' - 40° 11' Latitud S y 70° 55' - 71° 40' Longitud O. En cada sitio se tomaron datos de posicionamiento geográfico y se realizó un censo poblacional. Se tomaron datos morfométricos de los árboles muestreados que fueron analizados a través de técnicas multivariantes.

Se coleccionaron yemas invernales de los árboles muestreados. Éstas fueron injertadas sobre portainjertos clonales mediante injertos de tipo "chip budding". Las plantas obtenidas de esta manera fueron conservadas en el Banco de Germoplasma de la EEA Alto Valle-INTA.

Se evaluó la diversidad genética de 105 de los individuos coleccionados en 23 poblaciones mediante sistemas isoenzimáticos, primers de secuencias amplificadas al azar (RAPD) y primers de repeticiones de secuencias simples (SSR). En general, los valores encontrados para las distintas medidas de variabilidad estudiadas resultaron menores a los reportados en otras poblaciones de manzanos silvestres.

Con respecto a la distancia genética entre los individuos, la misma no permitió establecer agrupamientos coincidentes con su población de origen. En concordancia con estos resultados, no se encontró correlación entre las distancias genéticas y las distancias geográficas de las poblaciones estudiadas. Las mismas mostraron diferenciación con respecto a las variedades cultivadas.

Se estudió la diversidad genética y su distribución en las distintas poblaciones a través de diferentes métodos e indicadores. Los diferentes abordajes metodológicos coincidieron en que la mayor parte de la variabilidad genética se encuentra dentro de las poblaciones. Esto se atribuyó al flujo génico durante el establecimiento de las poblaciones, principalmente a través de semilla. Sin embargo, la proporción de diversidad genética entre las distintas poblaciones no es despreciable y alertaría sobre un proceso incipiente de fragmentación.

La falta de una estructuración evidente en el agrupamiento de los individuos, la ausencia de correlación entre las distancias genéticas y geográficas, y la ubicación de los sitios relevados indicarían un fuerte factor antrópico en la dispersión de los manzanos en el área de estudio.

El germoplasma colectado y conservado puede considerarse representativo de las poblaciones silvestres cordilleranas y diferente al de las principales variedades cultivadas.

Abstract

Apple tree (*Malus x domestica* Borkh.) is one of the most important fruit species. Argentina is one of the leading apple producers of southern hemisphere.

Since its origin in central Asia, this species has undergone a major expansion and selection in response to the interests of farmers and breeding programs.

The main worldwide varieties are very few; consequently the species has experienced genetic erosion in recent decades. In order to increase genetic variability it has been proposed to increase the number of accessions of primitive cultivars and wild types in genebanks, ensuring their availability for their incorporation into breeding programs or for direct use.

The aim of this study was to collect and preserve wild apple trees from the Andean mountain region, determine the genetic variability of the materials collected through isozyme and molecular markers and assess the distribution of genetic variability in populations.

Sixty-eight populations of wild apple trees were surveyed in an area between 38° 50'- 40°11' S Latitude and 70° 55'- 71° 40' W Longitude. In each population geographical positioning data and a census of trees were taken. Morphometric data from the sampled trees were analyzed through multivariate techniques.

Winter buds were collected from sampled trees. These buds were grafted on clonal rootstock by "chip budding" grafting. The obtained plants were conserved in the Germplasm Bank of INTA EEA Alto Valle.

Genetic diversity of 105 individuals collected from 23 populations was assessed using isozyme systems, primers random amplified sequences (RAPD) and primers of simple sequence repeats (SSR). The values found for the different studied measurements of variability were lower than those reported in other populations of wild apple trees.

Respect to the genetic distance between individuals, this does not allow to establish groupings matching with its population of origin. Consistent with these results, no correlation was found between genetic distances and geographical distances. Wild populations showed differentiation from the cultivated varieties.

Genetic diversity and its distribution in different populations were studied through different methods and indicators. The different methodological approaches concurred in finding most of the genetic variability within populations. This may be due to the presence of gene flow during the establishment of populations, mainly through seeds. However, the proportion of genetic diversity among different populations is not negligible and aware about an incipient process of fragmentation.

The lack of a clear structure in the grouping of individuals, the absence of correlation between genetic distances and geographic location of the surveyed sites indicate a strong anthropic factor in the spread of apple trees in the study area.

The germplasm collected and stored can be considered representative of wild populations and different from the germplasm present in the main cultivated varieties.

Bibliografía

Acquaah, G. 1992. Practical protein electrophoresis for genetic research. Dudley T. (Ed). Dioscorides Press, Portland, Oregon, USA. Chapter 3: Starch gel electrophoresis, 31-47.

Allendorf, F.W.; Luikart, G. 2007. Conservation and the Genetics of Populations. Blackwell Publishing, Cambridge, MA. 642 pp.

Aslantas, R.; Karakurt H. 2009. The effects of altitude on stomata number and some vegetative growth parameters of some apple cultivars. Research Journal of Agriculture and Biological Sciences, 5(5): 853-857.

Austerlitz, F.; Mariette, S.; Machon, N.; Gouyon, P.H.; Godelle, B. 2000. Effects of colonization processes on genetic diversity: differences between annual plants and tree species. Genetics 154: 1309–1321.

Balloux, F. 2004. Heterozygote excess in small populations and the heterozygote-excess effective population size. Evolution 58(9):1891-900.

Balzarini, M.; Di Rienzo, J. 2003. Info-Gen: Software para análisis estadístico de datos genéticos. Facultad de Ciencia Agropecuarias. Universidad Nacional de Córdoba. Argentina.

Balzarini, M.G.; Gonzalez, L.; Tablada, M.; Casanoves, F.; Di Rienzo, J.A.; Robledo C.W. 2008. Manual del Usuario, Editorial Brujas, Córdoba, Argentina.

Bandieri, S. 2005. Historia de la Patagonia. Ed: Sudamericana, Buenos Aires. 448p.

Bannister, M. H. 1965. Variation in the breeding system of *Pinus radiata*. En: Baker, H. G., and Stebbins, G. L. (Ed.), The Genetics of Colonising Species. Academic Press: 353-374.

Barden, J.A.; Nielsen, G.H. 2003. Selecting the orchard site, site preparation and orchard planning and establishment. En: Ferree, D C. (Eds). Apples: Botany, Production

and Uses. Edited by Ohio State University, USA; I Warrington, HortResearch, New Zealand. pp 237-266.

Baric, S.; Wagner, J.; Storti, A.; Dalla Via, J. 2011. Application of an extended set of microsatellite Dna markers for the analysis of presumed synonym cultivars of apple. *Acta Hort* 918:303–308.

Barrett, S.C.H.; Husband, B.C. 1990. The genetics of plant migration and colonization. En: *Plant population genetics, breeding and genetic resources* (Brown AHD, Clegg MT, Kahler AL, and Weir BS, eds). Sunderland, Massachusetts: Sinauer 254-277.

Bartish, I.; Jeppsson, N.; Nybom, H. 1999. Population genetic structure in the dioecious pioneer plant species *Hippophae rhamnoides* investigated by RAPD markers. *Molecular Ecology* 8: 791-802.

Batlle, I.; Alston, F. H.; Evans, K. M. 1995. The use of the isoenzymic marker gene *Got-1* in the recognition of incompatibility S alleles in Apple. *Theoretical and Applied Genetics* vol. 90 (2): 303 – 306.

Biedma, J. M. 1990. *Crónica histórica del Lago Nahuel Huapi*. 2da. Ed. Emecé Editores. Buenos Aires.

Botstein, D.; White, R. L.; Skolnick, M.; Davis, R. W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* 32:314-331.

Bran, D.; Ayesa, J.; López, C. 2002. *Áreas Ecológicas de Neuquén*. Informe INTA-EEA Bariloche. Comunicación Técnica de Relevamiento Integrado N° 70.

Broothaerts, W.; Van Nerum, I.; Keulemans, J. 2004. Update and review of the incompatibility (S-) genotypes of the apple cultivars. *Hortscience* 39 (5): 943-947.

Brown, A.H.D.; Briggs, J.D. 1991. Sampling strategies for genetic variation in ex situ collections of endangered plant species. In *Genetics and conservation of rare plants*, pp. 99–122. Eds. Falk D.A., Holsinger K.E. Oxford University Press, New York.

Brys, R.; Jacquemyn, H.; Endels, P. 2004. Reduced reproductive success in small populations of the selfincompatible *Primula vulgaris*. *J. Ecol* 92:5–14.

Bussell, J.D. 1999. The distribution of random amplified polymorphic DNA (RAPD) diversity amongst populations of *Isotoma petraea* (Lobeliaceae). *Molecular Ecology* 8, 775-789.

Büttner, R.; Fischer, M.; Forsline, P.L.; Geibel, M.; Ponomarenko, V.V. 2004. Gene banks for the preservation of wild apple genetic resources. *Journal of Fruit Ornamental Plant Research* 12, 99–104.

Cabrera, A. L. 1976. Regiones Fitogeográficas Argentinas. *Enciclopedia Argentina de Agricultura y Jardinería*. (2da ed). Tomo II, Fas 1. Ed. ACME, Buenos Aires. 85 pp.

Carrera, A. 2001. Marcadores isoenzimáticos como estimadores de variabilidad genética en germoplasma argentino de girasol. Tesis Doctoral. UNS.

Clausen, A.M.; Ferrer, M.E.; Formica, M.B. 2008. Situación de los recursos fitogenéticos en la Argentina. II Informe Nacional, 1996-2006. Ed: INTA, Córdoba, Argentina.

Clement, C. 2005. Fruits. En: Sir Prance G and Nesbitt M, editors. *The cultural history of plants*. Routledge, Madison Avenue, New York. p. 77-96.

Coart, E.; Van Glabeke, S.; De Loose, M.; Larsen, A.S.; Roldán-Ruiz, I. 2006. Chloroplast diversity in the genus *Malus*: new insights into the relationship between the European wild apple (*Malus sylvestris* (L.) Mill.) and the domesticated apple (*Malus domestica* Borkh.). *Molecular Ecology* 15: 2171-2182.

Coart, E.; Vekemans, X.; Smulders, M.J.M.; Wagner, I.; van Huylenbroeck, J.; van Bockstaele, E.; Roldán-Ruiz, I. 2003. Genetic variation in the endangered wild apple (*Malus sylvestris* (L.) Mill.) in Belgium as revealed by amplified fragment length polymorphism and microsatellite markers. *Molecular Ecology* 12:845–857.

Cornuet, J.M.; Luikart, G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, 144: 2001–2014.

Culley, T.M.; Grubb, T.C. 2003. Genetic effects of habitat fragmentation in *Viola pubescens* (Violaceae), a perennial herb with chasmogamous and cleistogamous flowers. *Molecular Ecology* 12:2919–293.

Currie, A.; Oraguzie, N. 2006. Simulation of genetic gain from two apple (*Malus x domestica* (Borkh.) breeding strategies. En: Mercer CF (ed) *Breeding for Success: Diversity in Action*. Proceedings of the 13th Australasian Plant Breeding Conference, Christchurch, 18–21 April. p 141.

Dawson, I. K.; Simons, A.J.; Waugh, R.; Powell, W. 1995. Diversity and genetic differentiation among subpopulations of *Gliricidia sepium* revealed by PCR-based assays. *Heredity* 74: 10-18.

De Mendieta, Y. 2005. *La Misión de Nahuel Huapi*. Ed: Carlos Alberto De Mendieta. San Carlos de Bariloche, República Argentina.

Di Rienzo, J.A.; Casanoves, F.; Balzarini, M.G.; Gonzalez, L.; Tablada, M.; Robledo C.W. 2008. *InfoStat*, versión 2008, Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.

Diaz, O.; Sun, G.L.; Salomon, B.; von Bothmer, R. 2000. Levels and distribution of allozyme and RAPD variation in populations of *Elymus fibrosus* (Schrenk) Tzvel. (Poaceae). *Genetic Research and Crop Evolution*, 47: 11–24.

Doligez, A.; Joly, H.I. 1997. Genetic diversity and spatial structure within a natural stand of a tropical forest tree species, *Carapa procera* (Meliaceae), in French Guiana. *Heredity* 79: 72-82.

Dunemann, F.; Kahnau, R.; Shmidt, H. 1994. Genetic relationships in *Malus* evaluated by RAPD ‘fingerprinting’ of cultivars and wild species. *Plant Breeding* 113: 150–159.

El-Kassaby, Y. A.; Ritland, K. 1996. Genetic variation in low elevation Douglas-fir of British Columbia and its relevance to gene conservation. *Biodiversity and Conservation* 5: 779-794.

Ellis, J.R.; Burke, J.M. 2007. EST-SSRs as a resource for population genetic analyses. *Heredity* 99: 125–132.

Ellstrand, N.C.; Elam, D.R. 1993. Population genetic consequences of small population size: implications for plant conservation. *Annual Review of Ecology & Systematics* 24: 217-242

Engelmann, F.; Engels, J.M.M . 2002. Technologies and Strategies for ex situ conservation. En: *Managing Plant Genetic Diversity*, (ed) Johannes M.M. Engels V. Ramanatha Rao, Anthony H.D. Brown y Michael T. Jackson. CABI Publishers. 487pp.

Excoffier, L.; Smouse, P.E.; Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131:479–491.

FAO. 2010. *The Second Report on the State of the World's Plant Genetic Resources for Food and Agriculture*. Rome. 370 pp.

Faostat. Consulta on line con fecha marzo de 2013. <http://faostat.fao.org>.

Ferrer, J.; Irisarri, J. 1990. Provincia del Neuquén. En: Moscatelli G. (Coord.) *Atlas de suelos de la República Argentina*. Tomo II. INTA, Buenos Aires. pp: 158-213.

Ferrer, M. E.; Clausen, A. M.; Menéndez Sevillano, M. del C.; Rosso, B. 2009. Red de recursos fitogenéticos del INTA. *Memorias del VII SIRGEALC* 565-567. Pucón, Chile.

Fondevila, A.; Moya, A. 1999. *Introducción a la genética de poblaciones*. Editorial Síntesis. Madrid, España. ISBN: 84-7738-691-9.

Ford, B.A.; McQueen, D.A.R.; Naczi, R.F.C.; Reznicek, A.A. 1998. Allozyme variation and genetic relationships in the *Carex willdenowii* complex (Cyperaceae). *Am. J. Bot.* 85: 546-552.

Forsline, P. L.; Dickson, E.E.; Dzhangaliev, A.D. 1994. Collection of wild *Malus*, *Vitis* and other fruit species genetic resources in Kazakhstan and neighboring republics. *HortScience* 29:433.

Forsline, P.L. 1995. Adding diversity to the national apple germplasm collection: collecting wild apples in Kazakstan. *New York Fruit Quarterly*. 3:3–6

Forsline, P.L. 2000. Procedures for collection, conservation, evaluation and documentation of *Malus* germoplasm. *Acta Hort*. 522:223-234.

Frankel, O.; Brown, A.; Burdon, J. 1995. The conservation of plant biodiversity. Cambridge University Press, Estados Unidos.

Frascaria, .N; Santi, F.; Gouyon, P.H. 1993. Genetic differentiation within and among populations of chestnut (*Castanea sativa* Mill.) and wild cherry (*Prunus avium* L.). *Heredity* 70:634-641.

Funbapa, 2012. Anuario 2012. <http://www.funbapa.org.ar/wp-content/uploads/2013/05/ANUARIO-MANZANA-PERA-2012.pdf>

Gardiner, S.E.; Bassett, H.C.M.; Madie, C.; Noiton, D.A.M. 1996. Isozyme, RAPD and RFLP markers used to deduce a putative parent for the “Braeburn” apple. *Journal of the American Society for Horticultural Science* 121:996–1001.

Garkava-Gustavsson L.; Kolodinsaka Brantesta A.; Sehic, J.; Nybom, H. 2008. Molecular characterisation of indigenous Swedish apple cultivars based on SSR and S-allele analysis. *Hereditas* 145:99-112.

Garkava-Gustavsson, L.; Antonius, K.; McDonagh, D.; Gallagher, T.; Nybom, H. 2011. Do we preserve unique apple germplasm? *Acta Hort*. 918:661-666.

Garkava-Gustavsson, L.; Nybom, H. 2007. Genetic diversity in a collection of apple (*Malus x domestica* Borkh.) cultivars as revealed by RAPD markers. *International Journal of Horticultural Science* 13:1–11.

Garkava-Gustavsson, L.; Persson, H.A.; Nybom, H.; Rumpunen, K.; Gustavsson, B.A.; Bartish, I.V. 2005. RAPD-based analysis of genetic diversity and selection of lingonberry (*Vaccinium vitis-idaea* L.) material for ex situ conservation. *Genetic Resources and Crop Evolution* 52:723–735.

Gasi, F.; Simon, S.; Posjskic, N; Kurtovic, M; Pejic, I. 2010. Genetic assessment of apple germplasm in Bosnia and Herzegovina using microsatellite and morphologic markers. *Scientia Horticulturae* 126:164-171.

Gept, P. 2006. Plant Genetic Resources Conservation and Utilization: The Accomplishments and Future of a Societal Insurance Policy. *Crop Sci.* 46:2278–2292.

Gepts, P. 2004. Crop domestication as a long-term selection experiment. In: *Plant Breeding Reviews*. J Janick (ed). Vol 24 Part 2. John Wiley & Sons. New York. pp:1-44.

Gharghani, A.; Zamani, Z.; Talaie, A.; Oraguzie, N.; Fatahi, R; Hajnajari, H.; Wiedow, C.; Gardiner, S. 2009. Genetic identity and relationships of Iranian apple (*Malus × domestica* Borkh.) cultivars and landraces, wild *Malus* species and representative old apple cultivars based on simple sequence repeat (SSR) marker analysis. *Genet Resour Crop Evol.* DOI 0.1007/s10722-008-9404-0.

Gianfranceschi, L.; Seglias, N.; Tarchini, R.; Komjanc, M.; Gessler, C. 1998. Simple sequence repeats for the genetic analysis of apple. *Theor Appl Genet* 96:1069–1076.

Giovannini, D.; Punelli, F.; Leone, A.; Liverani, A.; Ranieri, M.; Faedi, W. 2011. Genetic diversity in ancient fruit tree germplasm from southern Italy. *Acta Hort.* 918:741-748.

Goodman, M.M. 1990. Genetic and germ plasm stocks worth saving. *Journal of Heredity* 81, 11–16.

Gottlieb, L.1981. Electrophoretic evidence and plant populations. *Progress in Phytochemistry* 7: 1-46.

Goulao, L.; Cabrita, L.; Oliveira, C.; Leitao, J. 2001. Comparing RAPD and AFLPTM analysis in discrimination and estimation of genetic similarities among Apple (*Malus domestica* Borkh.) cultivars. *Euphytica* 119:259-270.

Goulao, L.; Oliveira, C.M. 2001. Molecular characterization of cultivars of apple (*Malus x domestica* Borkh.) using microsatellite (SSR and ISSR) markers. *Euphytica* 122:81-89.

Gower, J. C. 1971. A general coefficient of similarity and some of its properties. *Biometrics*, 27 (4):857-874.

Gross, B.L; Henk, A.D; Forsline, P.L; Richards, C.M. y Volk, G.M. 2012. Identification of interspecific hybrids among domesticated apple and its wild relatives. *Tree Genetics & Genomes* 8:1223-1235. DOI 10.1007/s11295-012-0509-4

Guarino, C.; Santoro, S.; De Simone, L.; Lain, O.; Cipriani, G. y Tesolin, R. 2006. Genetic diversity in a collection of ancient cultivars of apple (*Malus x domestica* Borkh.) as revealed by SSR-based fingerprinting. *Journal of Horticultural Science & Biotechnology* 81 (1):39-44.

Guilford, P.; Prakash, S.; Zhu, J.M.; Rikkerink, E.; Gardiner, S.; Basset, H.; Forster, R. 1997. Microsatellites in *Malus x domestica* (apple) abundance, polymorphism and cultivar identification. *Theor Appl Genet* 94:249-254.

Guo, S.W.; Thompson, E.A. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* 48:361-372.

Hammer, K.; Arrowsmith, N.; Gladis T. 2003. Agrobiodiversity with emphasis on plant genetic resources. *Naturwissenschaften* (2003) 90:241-250.

Hamrick, J. L.; Godt, M. J. W. and Sherman-Broyles, S. L. 1992. Factors influencing levels of genetic diversity in woody plant species. *New For.* 6:95-124.

Hamrick, J.L. y Godt, M.J. 1989. Allozyme diversity in plant species. En: Brown, A.H.D.; Clegg, M.T.; Kahler, A.L. y Weir B.S. Eds. *Plant Population Genetics, Breeding, and Genetic Resources*, pp.43-63, Sinauer Associates, Inc Publ. Sunderland, MA.

Harlan, J.R. 1992. Crops and Man. American Society of Agronomy, Madison, Wisconsin. 284 pp.

Harris, S.A.; Robinson, J.P. and Juniper, B.E. 2002. Genetic clues to the origin of the apple. Trends in Genetics 18(8): 426-430.

Hartl, D.L.; Clark, A.G. 1997. Principles of population genetics. 3rd edn. Sinauer, Sunderland.

Hemmat, M.; Weeden, N.F.; Manganaris, A.G. and Lawson D.M. 1994. Molecular marker linkage map in Malus. Journal of Heredity 85:4-11.

Hoisington, D.; Khairallah, M.; Gonzalez-de-León. 1994. Laboratory Protocols, CIMMYT, 2nd Edition, Mexico. DF: CIMMYT.

Hokanson, S.C.; Lamboy, W.F.; Szewc-Mc Fadden, A.K.; Mc Ferson, J.R.. 2001. Microsatellite (SSR) variation in a collection of Malus (apple) species and hybrids. Euphytica 118, 281-294.

Hokanson, S.C.; McFerson, J.R.; Forsline, P.L. and Lamboy, W. F. 1997. Collecting and managing wild Malus germplasm in its center of diversity. Hortscience 32(2):173-176

Hokanson, S.C.; Swezc-McFadden, A.K.; Lamboy ,W.F.; McFerson, J.R. (1998). Microsatellite (SSR) markers reveal genetic identities, genetic diversity and relationships in a Malus x domestica borkh. core subset collection. Theoretical and Applied Genetics, 97, 671-683.

Huff, D.R.; Peakall, R.; Smouse, P.E .1993. RAPD variation within and among natural populations of outcrossing buffalograss [Buchloë dactyloides (Nutt.) Engelm]. Theor Appl Genet 86:927-934.

InfoStat, versión 2008. 2008. Manual del Usuario. Grupo InfoStat, FCA, Universidad Nacional de Córdoba. Primera Edición, Editorial Brujas Argentina.

Itoiz, R.; Royo, B. Isoenzymatic variability in an apple germplasm bank. Genet Resour Crop Ev. 2003; 50:391-400.

Janick, J. 2005. The origin of fruits, fruit growing, and fruit breeding. *Plant Breeding Rev.* 25:255-320.

Janick, J.; Cummins, J.N.; Brown, S.K. and Hemmat, M. 1996. Apples. In: Janick J. and Moore J.N. (Eds.) *Fruit breeding, Volume I: Tree and tropical fruits*. John Wiley and Sons, Inc., New York, pp 1-77.

Johnson, R.S. and Lakso, A.N. 1985. Relationships between stem length, leaf area, stem weight, and accumulated growing degree-days in apple shoots. *Journal of the American Society for Horticultural Science* 110, 586–590.

Jordano, P. 2001. Conectando la ecología de la reproducción con el reclutamiento poblacional de plantas leñosas Mediterráneas. Págs. 183-211 en R. Zamora and F. Pugnaire, editores. *Aspectos ecológicos y funcionales de los ecosistemas mediterráneos*. Consejo Superior de Investigaciones Científicas, Madrid.

Jordano, P. and Godoy, J.A. 2000. RAPD variation and population genetic structure in *Prunus mahaleb* (Rosaceae), an animal-dispersed tree. *Molecular Ecology* 9:1293–1305.

Juniper, B. E.; Watkins, R.; Harris, S.A. 1998. The origin of the apple. *Acta Horticulturae* 148:27-33.

Juniper, B.E. and Mabberley, D.J. 2006. *The Story of the Apple*. Timber Press, Oregon.

Kery, M.; Matthies, D.; Spillmann, H.H. 2000. Reduced fecundity and offspring performance in small populations of the declining grassland plants *Primula veris* and *Gentiana lutea*. *J Ecol* 88:17–30.

Khoshbakht, K.; Hammer, K. 2005. Savadkouh (Iran) – an evolutionary centre for fruit trees and shrubs. *Genetic Resources and Crop Evolution* 00:1–11.

Koller, B.; Lehmann, A; McDermott, J.M. and Gessler, C. 1993. Identification of apple cultivars using RAPD markers. *Theor Appl Genet* 85:901–904.

Korban, S. S. and Skirvin, R. M. 1984. Nomenclature of the cultivated apple. *HortScience* 19:177-180.

Krause, S.; Hammer, K.; Buerkert, A. 2007. Morphological biodiversity and local use of the Himalayan pear (*Pyrus pashia*) in Central Bhutan. *Genet Resour Crop Evol* 54:1245–1254.

Kron, P.; Husband, B.C.; Keban, P.G. 2001a. Across- and along-row pollen dispersal in high-density apple orchards: insights from allozyme markers. *J. Hortic. Sci. Biotechnol.* 76, 286–294.

Kron, P.; Husband, B.C.; Keban, P.G.; Belaoussoff, S. 2001b. Factors affecting pollen dispersal in high-density apple orchards. *HortScience* 36, 1039–1046.

Kumar, S.; Volz, R.; Alspach, P.; Bus, V. 2010. Development of a current apple breeding programme in New Zealand: a synthesis of results, and a proposed revised breeding strategy. *Euphytica* 173: 207-222.

Kyndt, T.; Assogbadjo, A.E.; Hardy, Olivier J.; Glele Kakai, R.; Sinsin, B.; Van Damme, P.; Gheysen, G. 2009. Spatial genetic structuring of baobab (*Adansonia digitata* L., Malvaceae) in the traditional agroforestry systems of West Africa. *American Journal of Botany*, 96(5): 950-957.

Lambooy, W.F.; Yu, J.; Forsline, P.L.; Weeden, N.F. 1996. Partitioning of allozyme diversity in wild populations of *Malus sieversii* L. and implications for germplasm collection. *J Am Soc Hort Sci* 121:982-987.

Larsen, A.S. and Kjaer, D.E. 2008. Pollen mediated gene flow in a native population of *Malus sylvestris* and its implications for contemporary gene conservation management. *Conservation Genetics*, Vol: 10:1637-1646. DOI: 10.1007/s10592-008-9713-z.

Larsen, A.S.; Asmussen, C.B.; Olrik, D.C.; Coart, E.; Kjær ED. 2006. Hybridization and genetic variation in Danish populations of European crab apple (*Malus sylvestris*). *Tree Genet Genomes* 2:86–97.

Larson, B.M.H.; Kevam, P.G.; Inouye, D.W. 2001. Flies and flowers: taxonomic diversity of anthophiles and pollinators. *The Canadian Entomologist* 133:439-465.

Lauretin, H. 2009. Data analysis for molecular characterization of plant genetic resources. *Genetic Resource and Crop Evolution*, v. 56, n. 2, p. 277-292.

Lauri, P.; Maguylo, K.; Trottier, K. 2006. Architecture and size relations: and essay on the apple (*Malus x domestica*, Rosaceae) tree. *American Journal of Botany* 93(3):357-368.

Lespinasse, Y. 1992. Le pommier. In: *Ame´lioration des espèces vègétales cultivèes*, A. Gallais et H. Bannerot (eds) INRA, Paris, 579-594. ISBN: 2-7380-0383-4

Liebhart, R.; Gianfranceschi, L.; Koller, B.; Ryder, C.D.; Tarchini, R.; van de Weg, E.; Gessler, C. 2002. Development and characterisation of 140 new microsatellites in apple (*Malus • domestica* Borkh.). *Mol Breed* 10:217-241.

Litt, M.; Luty, J.A. 1989. A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *Am. J. Hum. Genet.* 44:398-401. 1989.

Loulakakis, K.; Roubelakis-Angelakis, K. y Kanellis, A. 1994. Regulation of glutamate Dehydrogenase and glutamine synthetase in avocado fruit during development and ripening. *Plant Physiology* 106:217-222.

Loveless, M.D. y Hamrick, J.L. 1984. Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics* 15:65-95.

Luby, J.; Forsline, P.; Aldwinckle, H.; Geibel, M. 2001. Silk Road Aples-Collection, Evaluation, and Utilization of *Malus sieversii* from Central Asia. *HortScience*, Vol. 36 (2): 225-231.

Luby, J.J.; Alspach, P.A.; Bus, V.G.M.; Oraguzie, N.C. 2002. Field resistance to fire blight in a diverse apple (*Malus* sp.) germplasm collection. *J Am Soc Hortic Sci* 127:245-253.

Luby, James. 2003. Taxonomic Classification and Brief History. En: Ferree, D C. (Eds). *Apples: Botany, Production and Uses*. Edited by Ohio State University, USA; I Warrington, HortResearch, New Zealand. pp 1-14.

Lynch, M. y Milligan, B. 1994. Analysis of population-genetic structure using RAPD markers. *Molecular Ecology* 3:91-99.

Mabberley, D.J.; Jarvis, C.E. and Juniper, B.E. 2001. The name of the apple. *Telopea* 9: 421-430.

Maguire, T.L.; Peakall, R.; Saenger, P. 2002. Comparative analysis of genetic diversity in the mangrove species *Avicennia marina* (Forsk.) Vierh. (Avicenniaceae) detected by AFLPs and SSRs. *Theor. Appl. Genet.* 104, 388-398.

Maki, M. 2003. Population genetics of threatened wild plants in Japan. *Journal of Plant Research* 116: 169-174.

Mariette, S.; Chagné, D.; Lézier, C. 2001. Genetic diversity within and among *Pinus Pinaster* populations: comparisons between AFLP and microsatellite markers. *Heredity*, 86, 469-479.

Mariette, S.; Lefranc, M.; Legrand, P.; Taneyhill, D.; Frascaria-Lacoste, N.; Machon, N. 1997. Genetic variability in wild cherry populations in France. Effects of colonizing processes. *Theor Appl Genet* , 94:904-908.

Marquat, R. y Chan, C. 1995. Identifying crabapple cultivars by isozymes. *J. Am. Soc. Hort. Sci.* 120(5):706-709.

Marshall, D.R. and Brown, A.D.H. 1975). Optimum sampling strategies in genetic conservation. In: Frankel, O.H. and Hawkes, J.G. (eds) *Crop Genetic Resources for Today and Tomorrow*. Cambridge University Press, New York, pp. 53-80.

Martínez, M.C; Helguera, M.; Carrera, A. 2010. Marcadores Moleculares. En: *Biotechnología y Mejoramiento Vegetal II. Parte I. Capítulo 5*. G Levitus, V Echenique, C Rubinstein, E Hopp, L Mroginski (eds). 647 p. INTA: 70-85.

Matsumoto, S.; Eguchi, T.; Bessho, H.; Abe, K. 2007. Determination and confirmation of S-RNase genotypes of apple pollinators and cultivars. *J Horticult Sci Biotechnol* 82:323-329.

Mecon, 2011. Complejo frutícola: manzana y pera. Serie "Producción Regional por Complejos Productivos". Mecon Ministerio Nacional de Finanzas Públicas. Pp 27. http://www.mecon.gov.ar/peconomica/docs/Complejo_pepitas.pdf

Menendez, R.A.; Fritts, R.; Larsen, F.E .1986. Identification of apple (*Malus domestica* Borkh.) rootstock cultivars by isozyme analysis. *J Am Soc Hort Sci* 111:933-937.

Mitton, J.B. 1993. Theory and data pertinent to the relationship between heterozygosity and fitness. In: Thornhill NW (ed) *The Natural History of Inbreeding and Outbreeding*, University of Chicago Press: Chicago.

Moreno, Francisco. 1969. *Viaje a la Patagonia Austral 1876-1877*, Buenos Aires, Solar/Hachette.

Moreno, Francisco. 1979. *Reminiscencias de Francisco P. Moreno*. Buenos Aires, Eudeba, [1906-1919].

Morgan, J.; Richards, A. 1993. *The book of apples*. Ebury Press, London, 304 pp. ISBN: 0-09-177759-3.

Mulcahy, D.L.; Cresti M.; Sansavini S.; Douglas G.C.; Linskens H.F.; Mulcahy G.B.; Vignani R. and Pancaldi M. 1993. The use of random amplified polymorphic DNAs to fingerprint apple genomes. *Sci Horticulturae* 54: 89-96.

Muster, George. 1963. *Vida entre los patagones. Un año de excursiones por tierras no frecuentadas desde el Estrecho de Magallanes hasta el Río Negro*, Buenos Aires, Solar-Hachette, [1873].

Nason, J. D. and J. L. Hamrick. 1997. Reproductive and genetic consequences of forest fragmentation: Two case studies of neotropical canopy trees. *J. Hered.* 88:264-276.

Nason, J.D.; Aldrich, P.R. y Hamrick, J.L. 1997. Dispersal and dynamics of genetic structure in fragmented tropical tree populations. Pag. 304-320. En *Tropical forest remnants: Ecology, management, and conservation of fragmented communities*. Laurance W. y Bierregaard Jr. R, Ed. University of Chicago Press, Chicago.

Nei, M. 1972. Genetic distance between populations. *American Naturalist* 106:283-392.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences, USA*, 70 (12, Parte 1), 3321-3323.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from small number of individuals. *Genetics* 89:583-590.

Nettancourt, N. D. 1977. *Incompatibility in angiosperms: monographs on theoretical and applied genetics*. Springer-Verlag, New York, New York, USA.

Niegel, J.E. 1997. A comparison of alternative strategies for estimating gene flow from markers. *Annual Review Ecology Systematics*. 28:105–128.

Noiton, D.; Alspach, P. 1996. Founding clones, inbreeding, coancestry, and status number of modern apple cultivars. *J. Amer. Soc. Hort. Sci.* 121:773-782.

Noiton, D.; Hofstee, P.; Alspach, P.; Brewer, L.; Howard, C. 1999. Increasing genetic diversity for apple breeding: A preliminary report. *Acta Hort.* 484:105-107.

Nybom, H.; Bartish, I. 2000. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. *Perspectives in Plant Ecology, Evolution and Systematics*, 3/2, 93–114.

Nybom, Hilde. 2004. Comparison of different nuclear DNA markers for estimating intraespecific genetic diversity in plants. *Molecular Ecology* 13:1147-155.

O'Rourke, D. 2003. *World Production, Trade, Consumption and Economic Outlook for Apples*. En: Ferree, D C. (Eds). *Apples: Botany, Production and Uses*. Edited by Ohio State University, USA; I Warrington, HortResearch, New Zealand. pp 15-29.

Oliveira, M. S. P.; Silva, K. J. D. Diferenciação genética entre procedências de açaizeiro por marcadores moleculares RAPD e SSR. *Revista Brasileira de Fruticultura*, v. 30, n. 02, p. 438-443, 2008.

Oraguzie, N. C.; Yamamoto, T.; Soejima, J.; Suzuki, T. and De Silva, H. N. 2005. DNA fingerprinting of apple (*Malus spp.*) rootstocks using Simple Sequence Repeats. *Plant Breeding* 124 (2), 197–202.

Oraguzie, N.C.; Mofstee, M.E.; Alspach, P.A.; Brewer, L.R.; Howard, C. 2000. Quantitative genetic studies in a recurrent selection program in apple. *Acta Hort.* 538: 105-107.

Ouborg, N.J.; Piquot, Y and Van Groendendael, J.M. 1999. Population genetics, molecular markers and study of dispersal in plants. *Journal of Ecology* 87:551-568.

Park, M.G.; Orr, M.C.; Danforth, B. N. 2010. The Role of Native Bees in Apple Pollination. *New York Fruit Quaterly* . Vol 18 . N. 1 . Sp 2010 21.

Peakall, R. y Smouse, P.E. 2006. GENALEX: genetix analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6, 288-295.

Peakall, R.; Smouse, P.E.; Huff , D.R. 1995. Evolutionary implications of allozyme and RAPD variation in diploid populations of Buffalograss (*Buchloë dactyloides* Nutt. Engelm.). *Mol Ecol.* 4:135–147

Pereira-Lorenzo, S.; Ramos-Cabrer, A.M.; Diaz-Hernandez, M.B. 2007. Evaluation of genetic identity and variation of local apple cultivars (*Malus × domestica* Borkh.) from Spain using microsatellite markers. *Genet Resour Crop Evol* 54:405–420. doi:10.1007/s10722-006-0003-7.

Pereira-Lorenzo, S.; Ramos-Cabrer, A.M.; González Diaz, A.J.; Diaz-Hernandez, M.B. 2008. Genetic assessment of local apple cultivars from La Palma, Spain, using simple sequence repeats (SSRs). *Scientia Horticulturae* 117:160-170.

Pereira-Lozano, S.; Ramos-Cabrer, A.; Ascasíbar- Erraste, J. 2003. Analysis of apple germplasm in northwestern Spain. *J. Am. Soc. Hort. Sci.* 128 (1):67-84.

Peterson, A.; Bartish, I.; Peterson, J. 2008. Effects of population size on genetic diversity, fitness and pollinator community composition in fragmented populations of *Anthericum liliago* L. *Plant Ecology* 198:101–110.

Phipps, J. B.; Robertson, K. R.; Smith, P. G.; Rohrer, J. R. 1990. A checklist of the subfamily Maloideae (Rosaceae). *Canad. J. Bot.* 68:2209–2269.

Piry, S.; Luikart, G.; Cornuet, J.M. 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *J Hered*; 90:502-503.

Rao, N. K. 2004. Plant genetic resources: Advancing conservation and use through biotechnology. *African Journal of Biotechnology* Vol. 3 (2):136-145.

Ravi, M.; Geethanjali, S.; Sameeyafarheen, F.; Maheswaran, M. 2003. Molecular marker based genetic diversity analysis in rice (*Oryza sativa* L.) using RAPD and SSR markers. *Euphytica* 133:243-252.

Richards, C.; Volk, G.; Reilly, A; Henk, A; Lockwood, D; Reeves, P.; Forsline, P. 2009. Genetic diversity and population structure in *Malus sieversii*, a wild progenitor species of domesticated apple. *Tree Genomics & Genomes* 5:339-347.

Riera, Rolando R. 1973. Dendrometría: Principios, descripción y uso del instrumental para edición forestal. En: Riera, Rolando R. *Apuntes de Silvicultura*. Universidad de Tucumán, República Argentina.

Rieseberg, L. and Soltis, D. 1989. Assessing the utility of isozyme number for determining ploidal level: Evidence from *Helianthus* and *Heliomeris* (Asteraceae). *Aliso* 12:277-286.

Rieseberg, L.; Beckstrom-Sternberg, S.; Liston, A.; Arias D. 1991. Phylogenetic and systematic inferences from chloroplast DNA and isozyme variation in *Helianthus* sect. *Helianthus* (Asteraceae). *Systematic Botany* 16:50-76.

Robinson, J.P.; Harris, S.A. and Juniper, B.E. 2001. Taxonomy of the genus *Malus* Mill. (Rosaceae) with emphasis on the cultivated apple, *Malus domestica* Borkh. *Plant Syst. Evol.* 226:35-58.

Rothlin, E. 1957. El manzano silvestre en la Patagonia. VII Congreso Frutícola Nacional y Exposición. Actas. Biblioteca del INTA- EEA Alto Valle.

Rousset, F. 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. *Mol. Ecol. Resources* 8:103-106.

Royo, J.B. and Itoiz, R. 2004. Evaluation of the discriminance capacity of RAPD, isoenzymes and morphologic markers in apple (*Malus x domestica* Borkh.) and the congruence among classifications. *Genetic Resources and Crop Evolution* 51(2):153-160.

Sakuri, K.; Brown, S.K. y Weeden, N.F. 2000. Self-incompatibility alleles of apple cultivars and advanced selections. *HortScience* 35, 116-119.

Samimy, C. y Cummins, J. N. 1992: Distinguishing apple rootstocks by isozyme banding patterns. *Horticultural science* 1:829-831.

Sansavini, S.; Donati, F.; Costa, F.; Tartarini, S. 2004. Advances in apple breeding for enhanced fruit quality and resistance to biotic stresses: New varieties for the European market. *Journal of Fruit Ornamental Plant Research, Special Edition*, 12:13-51.

Scarascia-Mugnozza, G.T. y Perrino, P. 2002. The History of ex situ Conservation and Use of Plant Genetic Resources. En: *Managing Plant Genetic Diversity*. Editores: Engels, J.; Rao, V. R.; Brown, A.; Jackson, M. Cabi Publishing. Wallingford, Inglaterra. 1-22.

Senasa. 2011. Anuario Estadístico 2011. Centro Regional Patagonia Norte, Senasa. 86 pp.

Slatkin, M. 1985. Gene flow in natural populations. *Annual Review of Ecology & Systematics* 16:393-430

Smouse, P. E. y V. L. Sork, V.L. 2004. Measuring pollen flow in forest trees: A comparison of alternative approaches. *Forest Ecology and Management* 197:21-38.

Smouse, P.E.; Peakall, R. 1999. Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity*. 82, 561-573.

Sokal, R. R. y Rohlf J. 1962. The comparison dendrogramas by objective methods. *Taxon* 11: 33-40.

StatSoft, Inc. 2005. STATISTICA (data analysis software system), version 7.1. www.statsoft.com.

Stephan, B. R.; Wagner, I. y Kleinschmit, J. 2003. EUFORGEN Technical Guidelines for genetic conservation and use for wild apple and pear (*Malus sylvestris* and *Pyrus pyraster*). International Plant Genetic Resources Institute, Rome, Italy.

Stoeckel, S.; Grange, J.; Fernández-Manjarres, J.; Bilger, I.; Frascaria-Lacoste, N. y Mariette, S. 2006. Heterozygote excess in a self-incompatible and partially clonal forest tree species-*Prunus avium* L. *Molecular Ecology* 15:2109-2118.

Stuber, C.; Goodman, M. 1983. Allozyme genotypes for popular and historically important inbred lines of corn, *Zea mays* L. US Department of Agriculture. agricultural research results. ARR-S-16/August.

Urrestarazu, J.; Laquidáin, M.J.; Miranda, C.; Santesteban, L.G. y Royo, J.B. 2011. Comparative analysis of the genetic diversity maintained in apple germplasm banks from northeastern Spain. *Acta Hort.* 918:655-660.

van Treuren R., Kemp H., Ernsting G., Jongejans B., Houtman H., Visser L., 2010. Microsatellite genotyping of apple (*Malus × domestica* Borkh.) genetic resources in the Netherlands: application in collection management and variety identification. *Genetic Resources and Crop Evolution*, 57: 853–865.

Vander Wall, S. 2002. Mastng in animal-dispersed pines facilitates seed dispersal. *Ecology* 83:12, 3508-3516.

Vavilov, N. 1951. Estudio sobre el origen de las plantas cultivadas. Ed: ACME Agency, Soc. Resp. Ltda, Buenos Aires Argentina. 185pp.

Venturi, S.; Dondini, L.; Donini, P.; Sansavini S. 2006. Retrotransposon characterisation and fingerprinting of apple clones. *Theor Appl Genet* 112:440–444

Vezub, J. 2005. Redes comerciales del País de las Manzanas. A propósito del pensamiento estructural de Guillermo Madrazo. Revista Andes N° 16, CEPIHA, Facultad de Humanidades, Universidad Nacional de Salta, 2005. pp. 167-198.

Viard, F.; El-Kassaby, Y. A. y Ritland, K. 2001. Diversity and genetic structure in populations of *Pseudotsuga menziesii* (Pinaceae) at chloroplast microsatellite loci. *Genome* 44:336-344.

Villarino, Basilio. Diario del piloto de la Real Armada, don Basilio Villarino. Del reconocimiento que hizo del Río Negro, en la costa oriental de Patagonia, el año de 1782. Buenos Aires, Imprenta del Estado. Primera ed: 1837. <http://www.cervantesvirtual.com/servlet/SirveObras/80216130656687729532279/index.htm>

Volk, G. M.; Richards, C. M.; Reilley, A. A.; Henk, A. D.; Forsline, P. L.; Aldwinckle, H. S. 2005. Ex situ Conservation of Vegetatively Propagated Species: Development of a Seed-based Core Collection for *Malus siervessii*. *Journal American Society Horticultural Science*, 130(2):203-210.

Weeden, N.F. y Lamb, R. C. 1987. Genetics and linkage analysis of 19 isozyme loci in apple. *J. Am. Soc. Hort. Sci.* 112 (5):865-872.

Weeden, N.F. y Lamb, R.C. 1985. Identification of apple cultivars by isozyme phenotypes. *J. Am. Soc. Hort. Sci.* 110:509-515.

Weir, B. S. y Cockerham, C. C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution*, 38:1358-1370.

Welsh, J. y McClelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res.* 18:7213-7218.

Wendel, J. y Weeden, N. 1989. Visualization and interpretation of plant isozymes. In: *Isozymes in plant biology*. D. Soltis y P. Soltis (Eds). Dioscorides Press. Portland, Oregon, USA. p. 5-44.

Westwood, M. N. y Roberts, A.N. 1970. The relationship between trunk cross-sectional area and weight of apple trees. *Journal of the American Society for Horticultural Science* 95:28–30.

White, T.W.; Adams, W.T. y Neale, D.B. 2007. *Forest Genetics*. Ed: Cabi Publishing.Oxford. 682 pp. ISBN 9780851990835.

Williams, J.G.; Kubelik, A.R.; Livak, K.J.; Rafalski, L.A. y Tingey, S.D. 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18:6531-6535.

Wright, S. 1951. The genetical structure of populations. *Annals of Eugenics*, 15:323-354.

Wright, S. 1978. *Evolution and the Genetics of Populations*. Vol. 4, Variability Within and Among Natural Populations. University of Chicago Press, Chicago.

Yeh, F.C. ;Yang , R.C.; Boyle, T.B.J.; Ye, Z.H. y Mao, J.X. 1999. POPGENE, the user friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada.

Yeh, F.C.; Yang, R-C.; Boyle, T. 1999. POPGENE version 1'31, Microsoft windows-based freeware for population genetic analysis. Edmonton: University of Alberta, Canada.

Zedan, H. 1995. Loss of plant diversity: a call for action. En: *Collecting Plant Genetic Diversity*. Ed: Guarino, L.; Ramanatha Rao, V; Reid, R. Cab International, UK.

Zhang, X.; Zhang, Y.; Yan, R.; Han, J.; Hong, F.; Wang, J.H.; Cao, K. 2010. Genetic variation of white clover (*Trifolium repens* L.) collections from China detected by morphological traits, RAPD and SSR. *African Journal of Biotechnology* 9: 3032-304.

Zhao, N.X.; Gao, Y.B.; Wang, J.L.; Ren A.Z. 2008. Population structure and genetic diversity of *Stipa grandis* P. Smirn, a dominant species in the typical steppe of northern China. *Biochemical Systematics and Ecology* 36: 1-10.

Zhou, Z.Q. y Li , Y.N. 2000. The RAPD evidence for the phylogenetic relationship of the closely related species of cultivated apple. *Genetic Resources and Crop Evolution* 47: 353–357.

Zohary, D; Hopf, M.; Weiss, E. 2012. *Domestication of plants in the Old World*, 4th edn. Oxford: Oxford University Press. N. Y. Estados Unidos.