Stephanie L. Greene
Karen A. Williams · Colin K. Khoury
Michael B. Kantar · Laura F. Marek
Editors

North American Crop Wild Relatives, Volume 1

Conservation Strategies

Springer

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Editors
Stephanie L. Greene
USDA, Agricultural Research Service
Center for Agricultural Resources
Research, National Laboratory
for Genetic Resources Preservation
Fort Collins, CO, USA

Karen A. Williams
USDA, Agricultural Research Service
Beltsville Agricultural Research
Center, National Germplasm
Resources Laboratory
Beltsville, MD, USA

Colin K. Khoury
USDA, Agricultural Research Service
Center for Agricultural Resources
Research, National Laboratory
for Genetic Resources Preservation
Fort Collins, CO, USA

International Center for Tropical
Agriculture (CIAT)
Cali, Colombia

Michael B. Kantar
Tropical Plant and Soil Science
University of Hawaii at Manoa
Honolulu, HI, USA

Laura F. Marek
Department of Agronomy/North Central
Regional Plant Introduction Station
Iowa State University
Ames, IA, USA

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This book is dedicated to the custodians of the diversity of wild plants in North America – past, present, and future

Foreword

Reinvesting in Crop Wild Relatives in North America

Nearly any place you hike a canyon, canoe a river, climb a mountain, wade a wet meadow, or weed a field in North America, you will come upon native plants that are close relatives of food, fiber, animal forage and feed, industrial oil, ornamental, and medicinal crops. On the fringes of muskegs from Hartley Bay in British Columbia to sites in northern California, you might come upon the Pacific crab apple (*Malus fusca* [Raf.] C. K. Schneid) around ancient fishing and gathering camps (Routson et al. 2012). In the watersheds of the St. Johns and Kissimmee Rivers in Florida, the vines of the rare Okeechobee gourd (*Cucurbita okeechobeensis* [Small] L. H. Bailey subsp. *okeechobeensis*) trail up into the branches of wild custard apples (*Annona glabra* L.) (Andres and Nabhan 1988; Nabhan 1989). Where I live and farm in Southern Arizona just north of the Mexican border, wild chiles (*Capsicum annuum* var. *glabriusculum* [Dunal] Heiser & Pickersgill) grow in desert canyons not far from wild grapes (*Vitis* L.), walnuts (*Juglans* L.), passion fruits (*Passiflora* L.), cassava (*Manihot* Mill), tomatillos (*Physalis* L.), and tepary beans (*Phaseolus acutifolius* A. Gray var. *acutifolius*) (Nabhan 1990; Nabhan 1991).

To many naturalists, these plants are but botanical curiosities, worthy of conservation without regard to historic or current human uses. But for crop geneticists and plant breeders, and a growing number of biodiversity conservationists, these species are especially worthy of protection, conservation, and evaluation because they may hold something of lasting value for the future of the way we live and eat on this planet.

These scientific and cultural values are exactly the reason why agricultural research and conservation management insights are not only so important but so timely. The chapters of this book represent the first comprehensive effort to assess wild crop genetic resources on our continent. Remarkably, this book arrives in our hands at just the moment in North American history when many of these plants are threatened by climate change yet also when these resources are most needed if future generations are to adapt.

The wildness in these plants confers distinct advantages not found in their domesticated cousins: tolerance to extremes of temperature and precipitation and resistance to the pests that lay waste to agricultural fields. This is why we are increasingly looking to the wild to strengthen our agriculture, particularly during this period of accelerated climate change. Farmers, orchardists, ranchers, and horticulturalists are already suffering from shifting and often heightened frequencies of drought, heat waves, catastrophic freezes, hurricanes, floods, and fires. And with the changes in these abiotic stressors come other biotic impacts to our farming systems and food security: previously unforeseen weeds, insect pests, and crop diseases that take a long-term toll on agricultural productivity and food safety.

During eras of political, economic, and environmental stress, humanity turns to consider a broader range of options than typically employed during "business as usual." This is one of those times – when agriculture is looking to draw upon a broader and deeper gene pool of crop genetic resources as a means to re-diversify and add resilience to the food plants that we depend on for survival. After decades of focusing on a relatively small genetic base of cultivated varieties for crop improvement, geneticists are now casting a much wider net, fortunately enabled by a broader portfolio of diagnostic techniques, micro-propagation practices, and biotechnologies used to select and transfer genes from wild relatives into food crops.

This is why the fact that forty-some crop wild relative species are included in the U.S. Fish and Wildlife Service's Endangered and Threatened Plant list is of great concern. To make matters worse, this list is likely to grow much larger (Rogers 2015). Analyses of North American seed plants facing extinction risks exacerbated by climate change and land use intensification (Zhang et al. 2016) would indicate that roughly 27% of the 4600 crop wild relatives documented to occur in the U.S. (Khoury et al. 2013) are likely to lose more than 80% of their habitat by the 2080s and will suffer a 50% retraction of their ranges.

As I read through the names on the current U.S. list of threatened and endangered crop wild relatives, I am struck by both their beauty and by the fragility of the plant species they represent:

- Texas wildrice (*Zizania texana* Hitchc.), an aquatic perennial with high allelic richness surviving along just a few stretches of the San Marcos River drainage of the Edwards Plateau in Texas
- The scrub plum (*Prunus geniculata* R. M. Harper) of Lake Wales Ridge in Florida, a small shrub with perfumed flowers valued by horticulturists as a showy and fragrant ornamental, closely related to the Chickasaw plum, with a fruit of probable hybrid origin that has been both culturally dispersed and cultivated for well over 150 years
- The Bakersfield prickly pear cactus [*Opuntia basilaris* Engelm. & J. M. Bigelow var. *treleasei* (J. M. Coult.) J. M. Coult. ex Toumey], with genes for drought resistance and production of compounds which protect against adult-onset diabetes, both of which desperately needed by farmers and consumers on our continent

- The puzzle sunflower (*Helianthus paradoxus* Heiser), a halophyte of the Pecos River in New Mexico and West Texas, which emerged from a chance hybrid of the common sunflower (*Helianthus annuus* L.) and the prairie sunflower (*Helianthus petiolaris* Nutt.) over 75,000 years ago, but is now far more salt tolerant than either of its parents, and most cultivated sunflower hybrids as well
- The Okeechobee gourd, a squash relative first described by John and William Bartram along the St. Johns River in Florida around the time of the Revolutionary War, which has barely survived the agricultural revolution that drained the Everglades for sugarcane production and diverted most rivers in Florida into croplands (Nabhan 1989)
- The Oahu cowpea (*Vigna o-wahuensis* Vogel), a rare perennial legume that has gone extinct on the very island in Hawaii where my daughter and grandson now live but which tenaciously hangs on for dear life in just seven small populations spread across four of the other Hawaiian Islands

I offer you these brief “personality profiles” to remind you that each of these valuable and endangered crop relatives has a distinctive character. The tasks of identifying, counting, tallying, mapping, monitoring, and managing the remaining populations of rare plant species on the verge of extinction are ever increasing. It is worth a moment of our time now and then to remember the complex ecological and human relationships surrounding each of these unique but declining plants.

It is important to remember that the contribution of wild relatives to crops is not a new phenomenon. In fact, these plants have naturally exchanged genes in traditional agricultural settings for millennia. We are all beneficiaries of such serendipitous crop diversification every time we sit down to eat a meal or drink a glass of wine or cider. They have been – and continue to be – our most useful “living library,” a set of manuals to help us maintain our food security (Gruber 2017, Khoury 2015, and in this volume).

Several of the chapters in this book point out the importance of recognizing that many crop relatives remain economic crops and cultural resources in their own right. Plant breeders do not necessarily need to “improve” some of these plants to make them acceptable to the public. For example, the fresh and dried fruits of wild chiltepín peppers (*Capsicum annuum* var. *glabriusculum*) sell for more than USD $80 per pound in much of the U.S. Southwest and northwestern Mexico. One pound of American wildrice from Minnesota streams and lakes that is hand-harvested and wood-parched by Native American foragers garners prices of up to USD $17 on Amazon. On the southern edges of the Chihuahuan Desert, consumers are willing to pay five to ten times more for a delicious semi-cultivated potato called papita güera (*Solanum cardiophyllum* Lindl) than for domesticated potatoes of exotic origin. Yet the anciently cultivated genotypes of this species are hardly if at all represented in most potato gene banks, including those in Mexico and the USA.

Wild apples (*Malus* Mill.) prized for their tartness and flavor are now included in hard ciders in the USA and Canada. Legally collected or propagated rare food crops like Price’s potato bean (*Apios priceana* B. L. Rob.), agaves (*Agave* L.), and cacti command high prices in horticultural trade. Wild prickly pears (*Opuntia* Mill.), pinyon nuts (*Pinus* L.), and ramps (*Allium tricoccum* Aiton) continue to attract almost as much attention from chefs and nutritional scientists as their cultivated counterparts do. Nevertheless, habitat fragmentation and other threats are diminishing foragers' access to these North American plants.

Beyond these direct uses, promising new applications of these plants are emerging from recent innovations in applied research. In Kansas, the Land Institute is newly domesticating perennial wild relatives of food crops, using intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & D. R. Dewey) and rosinweed (*Silphium integrifolium* Michx.), a distant relative of sunflower, in their prairie-adapted polycultures (Dehaan et al. 2016; Van Tassel et al. 2017). In Missouri, botanists associated with the Missouri Botanical Garden and St. Louis University are evaluating wild relatives of commercially important fruit tree crops for development in their own right both as sources of food and as rootstocks, due to their hardiness and resistance to emerging insect pests and plagues (Allison Miller, pers. com).

In Illinois, integrated pest management teams have experimented with the native buffalo gourd (*Cucurbita foetidissima* Kunth) as a trap crop grown on the edges of squash and pumpkin fields to reduce larval damage to these crops and increase pollination efficiencies (Metcalf et al. 1980; Metcalf et al. 1982). In Arizona, our ecological research in the first in situ reserve for crop wild relatives in the USA [in Coronado National Forest] allowed us to determine how capsaicinoids and other secondary metabolites serve as "directed" chemical defenses against *Fusarium* fungi, insect pests, and seed-predating rodents in wild chiltepín peppers (Tewksbury and Nabhan 2001; Eich 2008). It may now be possible to differentially select and use the various capsaicinoids in the wild chile pepper arsenal for the discouragement of grain storage pests, prevention of fungal contamination of seeds, treatment of shingles, reduction of blood serum cholesterol and glucose, and management of attention-deficit disorders (Eich 2008; Barchenger and Bosland, this volume).

Thus, crop wild relatives are extremely valuable genetic resources, yet they also offer us their colorful and meaningful natural histories – stories of survival, if you will, of a more diverse portfolio of plants still available to humanity.

Thankfully, as many of the chapters in this book document, the conservation and use of wild relatives is getting more serious traction, with national and international initiatives looking to make a significant impact in the coming years. But these efforts are the tip of the iceberg of what is needed. As several contributions in this volume affirm, we must continue to invest in sufficiently supporting every link in the wild relative-food crop supply chain – from in situ conservation of natural habitats in national parks and biosphere reserves to ex situ seed banks, botanical gardens, and plant restoration efforts – if the entire supply and delivery system is to function for the future. It is not enough for land grant universities to invest millions in molecular biology laboratories if they end up closing down herbaria and cutting budgets of campus arboreta and experimental farms in the process. As Harvard conservation biologist E.O. Wilson once quipped, it is the "non-sexy" and more descriptive sciences of systematics, ethnobotany, biogeography, and seed storage physiology that have gotten us to where we are today.

These recently emerged opportunities will not bear fruit if our funding sources for habitat conservation and landscape management, for basic biology and seed banking, and for horticultural innovation and biomedical research focus only on the last few links of the wild relative-crop commodity supply chain. We not only need to diversify the genetic base of our food supply, we also need to diversify and sustain the many forms of conservation, restoration, and scholarly inquiry which together ensure access to these crop genetic resources.

Collectively, the chapters in this remarkable book provide a valuable overview of the best information and practices needed to safeguard and wisely use North America's crop wild relatives. Detailing the species native and naturalized in the continent and related to important food, fiber, animal forage and feed, industrial oil, ornamental, and medicinal crops, the authors outline their potential for use and highlight the conservation needs for the species. In bringing together for the first time this information from across the broad North American region, including Canada, Mexico, and the USA, the book provides access to critical conservation information for well over 600 promising plants. As this landmark volume attests, these plants are essential elements of North America's natural and cultural heritage. This book becomes the model for advancing the efforts needed to better care for this heritage for present and future generations. It provides us with operating instructions for wisely managing "our living library."

Tucson, AZ, USA Gary Paul Nabhan

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Preface

Wild plants useful to food and agriculture occupy a niche frequently outside the realm of both agricultural and natural resource professionals. The agricultural community tends to focus on a handful of domesticated species, while the natural resource community emphasizes legislatively regulated taxa (i.e., species that are rare, endangered, indicators of ecosystems such as wetlands, or wild species used for timber or revegetation).

The increasing challenges to food and agricultural production due to climate change, added to the ever-present biotic and abiotic stresses, are likely to make crop wild relatives and other useful wild plants more of a priority to both communities. And the combined efforts of both communities will be critical to locating, conserving, and making available these invaluable species to support agricultural production and food and nutritional security for future generations.

The purpose of this two-volume book is to highlight the most important wild plant genetic resources that grow in North America. We define these resources as wild species with relevance for agriculture. These include the wild plant populations from which domesticated varieties evolved, crop wild relatives that can be used to improve contemporary crops, wild species that have a record of use by people, and any other wild species with potential for future crop development. Most of the species covered are native, but a few are introduced taxa that have become naturalized in the region. A thorough understanding of the species that occupy North America, including their distributions, potential value to agriculture, and conservation statuses and needs, will give agricultural and conservation communities the basic knowledge they need to take steps to conserve our natural heritage of useful wild plants.

The overarching goal of this book is to help ensure that these valuable but overlooked species continue to persist, both in their natural habitats and in gene banks, where they can be made available as resources to address compounding agricultural and nutritional challenges. This book is authored by a broad range of experts working diligently to explore, protect, celebrate, and use crop genetic resources. They have come together to compile the latest information on the most important North American wild useful plants. This book focuses on Canada, the USA, and Mexico, three countries whose combined area covers most of the continent.

The first volume of the book covers topics relevant to the conservation of all wild plant genetic resources, while the second volume focuses on specific crops and their related wild taxa. Volume One: Conservation Strategies begins by reviewing efforts, challenges, and opportunities to conserve food and agriculturally important wild species from a national perspective. The first part provides not only a broad overview of important crop wild relatives and wild utilized species in Canada, Mexico, and the USA but also a description of the agencies and institutes focused on conserving these plants, as well as the conservation and use policies followed by each country. This section concludes with a chapter that presents Native American tribal perspectives in the USA, providing a glimpse into the management and regulation of plant genetic resources by Indigenous peoples through a set of case studies of several tribal governments.

The second part of Volume One discusses various aspects of wild plant genetic resource conservation methodologies. Managing genetic resources of wild plants involves additional considerations beyond those required for domesticated crops. Likewise, wild genetic resource conservation differs from managing plant species that are rare and endangered in that a greater emphasis is placed on ex situ activities, including storage and seed regeneration, that ensure that these resources are available for use by plant breeders and the scientific community. This section is organized in a logical sequence that begins with a chapter on current and emerging frameworks on defining wild genetic resources, as well as a chapter on threat assessments. Two subsequent chapters cover sampling strategies and collecting practicalities, followed by chapters that discuss aspects of gene banking wild species, including storage and seed increase. Volume One concludes with a chapter that discusses public education and outreach opportunities for crop wild relatives.

There are over 20,000 plant species in North America, and all deserve a chance to thrive. However, a small fraction of these are distinguished by their potential to support food and agricultural production, either because they are resources that can be used to breed more productive crops or because they have commercial or cultural value when used directly. Many of these species are common, even weedy, and are easily overshadowed by rare or endangered plants. Nevertheless, because of their real or potential importance to our food and agriculture, they deserve to be recognized, celebrated, conserved, and made available for use.

The Editors would like to acknowledge and thank the authors for the extensive work they have done to compile, organize, and write their chapters. We would also like to acknowledge the contribution and insights provided by peer reviewers of each chapter.

Fort Collins, CO, USA	Stephanie L. Greene
Beltsville, MD, USA	Karen A. Williams
Fort Collins, CO, USA	Colin K. Khoury
Honolulu, HI, USA	Michael B. Kantar
Ames, IA, USA	Laura F. Marek

Contents

Part I Conservation in North America

1 Wild Plant Genetic Resources in North America: An Overview 3
Stephanie L. Greene, Colin K. Khoury, and Karen A. Williams

2 Genetic Resources of Crop Wild Relatives: A Canadian Perspective 33
Axel Diederichsen and Michael P. Schellenberg

3 Crop Wild Relatives in Mexico: An Overview of Richness, Importance, and Conservation Status 63
J. Fernando De La Torre S., Rosalinda González S., E. Judith Cruz G., J. Manuel Pichardo G., Martín Quintana C., Aremi R. Contreras T., and Jorge Cadena I.

4 Conservation of Crop Wild Relatives in the USA 97
Karen A. Williams and Stephanie L. Greene

5 Working with Indigenous Communities: The Original Caretakers of Crops and Crop Wild Relatives 155
Scott M. Herron

Part II Conservation Strategies

6 The Gene Pool Concept Applied to Crop Wild Relatives: An Evolutionary Perspective 167
Richard E. Miller and Colin K. Khoury

7 Conservation Status and Threat Assessments for North American Crop Wild Relatives 189
Anne L. Frances, Adam B. Smith, and Colin K. Khoury

8 Sampling Wild Species to Conserve Genetic Diversity 209
Sean Hoban, Gayle Volk, Kanin J. Routson, Christina Walters, and Chris Richards

9 Practicalities of Collecting Wild Plants in North America: Insights from the United States 229
Laura F. Marek

10 Genebank Conservation of Germplasm Collected from Wild Species 245
Christina Walters, Christopher M. Richards, and Gayle M. Volk

11 Practical Considerations for Increasing Seed Samples of Wild Species 281
Barbara C. Hellier

12 Public Education and Outreach Opportunities for Crop Wild Relatives in North America 311
Tara Moreau and Ari Novy

Appendix 1 Map Methods and Occurrence Data Sources 325

Common Name Index 335

Scientific Name Index 339

Reviewers

Tabare Abadie DuPont Pioneer, Johnston, IA, USA

Dani Ballesteros Kew Royal Botanical Gardens, Richmond, England, UK

Vicki Bradley USDA Agricultural Research Service, Pullman, Washington, USA

Peter Bretting USDA Agricultural Research Service, Beltsville, MD, USA

Jacques Cayouette Agriculture and Agri, food Canada, Ottawa, ON, Canada

Neil Cox The International Union for Conservation of Nature, Washington DC, USA

Denise Culver Colorado National Heritage Program, Fort Collins, CO, USA

Luigi Guarino Crop Trust, Bonn, Germany

Edward Guerrant Portland State University, Portland, OR, USA

Eric N. Jellen Brigham Young University, Provo, UT, USA

Amanda C. Martin The Kochi Prefectural Makino Botanical Garden, Kochi City, Japan

Allison Miller Saint Louis University, St Louis, MO, USA

Melanie Schori USDA Agricultural Research Service, Beltsville, MD, USA

Susan Stieve The Ohio State University, Columbus, OH, USA

Abbreviations

AAFC	Department of Agriculture and Agri-Food Canada
ABS	Access and Benefit Sharing
ABSA	Access and Benefit Sharing Agreement
AFLP	Amplified fragment length polymorphism
AHLV	American hop latent virus
AHPA	American Herbal Products Association
AMJB	Mexican Association of Botanical Gardens
ANSI	American National Standards Institute
ANSM	Universidad Autónoma Agraria Antonio Narro
AOSCA	Association of Official Seed Certifying Agencies
APGA	American Public Gardens Association
ApMV	Apple mosaic virus
ARS	Agricultural Research Service
ASU	Arizona State University
ATBI	All Taxa Biodiversity Inventory
AZ	Arizona
BCMV	Bean common mosaic virus
BGCI	Botanic Gardens Conservation International
BIA	Bureau of Indian Affairs
BLM	Bureau of Land Management
BONAP	Biota of North America Program
BP	Before present
BP&P	Bayesian phylogenetics and phylogeography
Bt	Bacillus thuringiensis
BTA	Boyce Thompson Arboretum
CA	California
CAM	Crassulacean acid metabolism
CAPS	Cleaved amplified polymorphic sequences
CBCN	Canadian Botanical Conservation Network
CBD	Convention on Biological Diversity
CBIF	Canadian Biodiversity Information Facility

CCC	Civilian Conservation Corps
CCD	Colony collapse disorder
CCGB	Canadian Clonal Genebank
CCN	Conservation Center Network
CDFW	California Department of Fish and Wildlife
CDI	National Commission for the Development of Indigenous Peoples
CEVAMEX	Valle de México Research Station
CFIA	Canadian Food Inspection Agency
CGC	Crop Germplasm Committee
CGN	Centre for Genetic Resources
CIAT	International Center for Tropical Agriculture
CICTAMEX	Scientific and Technological Research Center of Avocado in the State of Mexico
CICY	Yucatan Center for Scientific Research
CIMMYT	International Maize and Wheat Improvement Center
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CLCuD	Cotton leaf curl disease
CMS	Cytoplasmic male sterility
CMV	Cucumber mosaic virus
CNHP	Colorado Natural Heritage Program
CNRG	National Genetic Resources Center
Co	County
CONABIO	National Commission for the Knowledge and Use of Biodiversity
CONANP	National Commission of Natural Protected Areas
COSEWIC	Committee on the Status of Endangered Wildlife in Canada
CP	Coefficient of Parentage
CP	Postgraduate College
CPB	Cartagena Protocol on Biosafety
CPC	Center for Plant Conservation
CPGR	Canadian Potato Genetic Resources
CPNWH	Consortium of Pacific Northwest Herbaria
CS	College Station Texas
CSIRO	Commonwealth Scientific and Industrial Research Organization
CSN	Counter Season Nursery
CWR	Crop wild relatives
DELEP	Desert Legume Program
DMI	Dobzhansky-Muller incompatibilities
DNA	Deoxyribonucleic acid
DNR	Department of Natural Resources
DOD	Department of Defense
DOE	Department of Energy
DOF	Official Gazette of the Federation
DOT	Department of Transportation

EC	Electrical conductivity
ECOS	Environmental Conservation Online System
ELISA	Enzyme-linked immunosorbent assay
EMCV	Mexican Plant Conservation Strategy
ENBioMex	National Biodiversity Strategy of Mexico
ENGOs	Environmental non-governmental organizations
ENM	Ecological niche model
ESA	Endangered Species Act
EUFGIS	European Information System on Forest Genetic Resources
FAO	Food and Agriculture Organization of the United Nations
FES-I	Faculty of Higher Education Iztacala
FGP	Frost grape polysaccharide
FNA	Flora of North America
FRRL	Forage and Range Research Laboratory
G	Global
GBIF	Global Biodiversity Information Facility
GBS	Genotyping by sequencing
GG	Germplasm Resource Information Network-Global database
GH	Greenhouse
GIS	Geographical Information Systems
GLIFWC	Great Lakes Indian Fish and Wildlife Commission
GLS	Gray leaf spot
GMP	Good Manufacturing Practice
GP	Genepool
GPS	Global Positioning System
GRIN	Germplasm Resource Information Network database
GS	Genome selection
GWAS	Genome-wide association study
Ha	Hectare
HPLC	High-performance liquid chromatography
HpLV	Hop latent virus
HpMV	Hop mosaic virus
HPTLC	High-performance thin-layer chromatography
HRT	Hormone replacement therapy
ICAMEX	Institute for Training, Research, and Development in Agriculture of the State of Mexico
ICAR	Indian Council on Agricultural Research
ICRISAT	International Crop Research Institute for the Semi-Arid Tropics
iDIGBio	Integrated Digitized Biocollections
IGRR	In Situ Genetic Resource Reserve
IICA	Inter-American Institute for Cooperation on Agriculture
ILDB	The International Lactuca Database
INDR	Indiana Department of Natural Resources
INIFAP	National Institute of Forestry, Agriculture and Livestock Research
INRA	French National Institute for Agricultural Research

INTA	Instituto Nacional de Tecnología Agropecuaria
IPGRI	International Plant Genetic Resources Institute
IPK	Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung
IPR	Intellectual property rights
ISO	International Standards Organization
ISSR	Inter-simple sequence repeat
ITIS	Integrated Taxonomic Information System
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture
IUCN	International Union for Conservation of Nature
KBIC	Keweenaw Bay Indian Community
LBOGM	Law on Biosafety of Genetically Modified Organisms
LFVV	Federal Law on Plant Varieties
LGCC	Law on Climate Change
LGCS	USDA Lactuca Germplasm Collection in Salinas
LGRCD	Lactuca Genetic Resources Collection at the University of California in Davis
MCMV	Maize chlorotic mosaic virus
Mha	Million hectares
MLN	Maize lethal necrosis
MLS	Multilateral system
MRC	Major resistance clusters
MRCA	Most recent common ancestor
MS	Flow injection mass spectrometry
MSB	Millennium Seed Bank
MSBP	Millennium Seed Bank Project
MT	Metric tons
MTA	Material Transfer Agreement
N	National
n/d	Not determined
NA	North America
NALPGRU	National Arid Land Plant Genetic Resources Unit
NARO	National Agriculture and Food Research Organization
NASS	National Agricultural Statistics Service
NBSAP	National Biodiversity Strategy and Action Plan
Nc	Census population size
NCGC	National Cotton Germplasm Collection
NCGR	National Clonal Germplasm Repository
NCGR-Davis	National Clonal Germplasm Repository in Davis, California
NCRPIS	North Central Regional Plant Introduction Station
Ne	Effective population size
NGRL	National Germplasm Resources Laboratory
NGS	Next-generation sequencing
NILs	Near isogenic lines
NKLP	Nagoya–Kuala Lumpur Protocol on Liability and Redress

NLGRP	National Laboratory for Genetic Resources Preservation
NLR	Nucleotide binding-leucine rich repeat receptor
NM	New Mexico
NMFS	National Marine Fisheries Service
NMR	Nuclear magnetic resonance
NOM-059	NOM-059-SEMARNAT-2010-Mexican standard that lists all threatened native wild species
NORGEN	North American Regional Network for Agricultural Research-Genetic Resources Task Force
NP	Nagoya Protocol
NPA	Natural Protected Areas
NPABS	Nagoya Protocol on Access and Benefit Sharing
NPGS	USDA, ARS National Plant Germplasm System
NPS	National Park Service
NRCS	USDA, Natural Resources Conservation Service
NS SEME	Novi Sad Institute of Field and Vegetable Crops
NS/S	Native Seeds/Search
NSL	United States Forest Service National Seed Laboratory
NUS	Neglected underutilized species
OPGC	Ornamental Plant Germplasm Center
OR	Oregon
OSU	Ohio State University
PacBio	Pacific Biosystems
PANREFI	National Plan of Action for the Conservation of Plant Genetic Resources for Food and Agriculture
PBLS	Pecan bacterial leaf scorch
PCA	Plant Conservation Alliance
PCB	Cartagena Protocol on Biosafety
PCN	Plant Conservation Network
PCR	Polymerase chain reaction
PEO	USDA, ARS Plant Exchange Office
PGRC	Plant Gene Resources of Canada
PGRFA	Plant Genetic Resources for Food and Agriculture
PI	Plant Introduction
PMC	USDA, Natural Resources Conservation Service Plant Material Center
PROCINORTE	North American Regional Network for Agricultural Research
PRV	Papaya ringspot virus
PVG	Pre-variety germplasm
PVP	US Plant Variety Protection
QTL	Quantitative trait loci
RAPD	Random amplified polymorphic DNA
RBG Kew	Royal Botanical Gardens Kew
RGC16	Family of nucleotide binding-leucine rich repeat receptor (NLR) proteins

RGC2	Resistance Gene Candidate 2
RMA	Research Marketing Act
RNA	Research Natural Area
RNA	Ribonucleic acid
S	Subnational
SAGARPA	Secretariat of Agriculture, Livestock, Rural Development, Fisheries and Food
SBBG	Santa Barbara Botanic Garden
SCP	Species Conservation Plan
SDBG	San Diego Botanic Garden
SDG	Sustainable Development Goals
SDM	Species distribution model
SEINet	Southwestern Environmental Information Network
SEMARNAT	Ministry of the Environment and Natural Resources
SID	Seed Information Database
SINAREFI	National Program of Plant Genetic Resources for Food and Agriculture
SMTA	Standard material transfer agreement
SNICS	National Seed Inspection and Certification Service
SNP	Single nucleotide polymorphisms
SNWA	Southern Nevada Water Authority
SOMEFI	Mexican Society of Plant Genetics
SOP	Standard operating procedure
SOS	Seeds of Success
SS	Sequence-tagged site
SSR	Simple sequence repeat
SW	Southwestern
SWD	Spotted wing drosophila
TES	Threatened, Endangered, and Sensitive Species
TEX	University of Texas
Tg	Glass transition temperature
TNC	The Nature Conservancy
TNPD	Texas Native Plants Database
TRAP	Target region amplification polymorphism
TRIPS	Trade-Related Aspects of Intellectual Property Rights
TVA	Tennessee Valley Authority
TX	Texas
UAAAN	Antonio Narro Agrarian Autonomous University
UACh	Chapingo Autonomous University
UBC-BG	University of British Columbia Botanical Garden
UC Davis V&E	The University of California, Davis Viticulture and Enology Department
UCBG	University of California Botanical Garden
UDG	University of Guadalajara
UGto	University of Guanajuato

UMA	Wildlife Conservation Management Units
UNAM	National Autonomous University of Mexico
UNCCD	United Nations Convention to Combat Desertification
UNESCO	United Nations Educational, Scientific and Cultural Organization
UNFAO	United Nations Food and Agriculture Organization
UNFCCC	United Nations Framework Convention on Climate Change
UPOV	International Union for the Protection of New Varieties of Plants
UPP	Useful Plants Project
URL	Uniform Resource Locator
US	United States
USA	United States of America
USD	United States Dollars
USDA	United States Department of Agriculture
USDA-ARS	United States Department of Agriculture – Agricultural Research Service
USDOI	United States Department of the Interior
USFS	United States Forest Service
USFWS	US Fish and Wildlife Service
USFWS	United States Fish and Wildlife Service
UT	Utah
UV	University of Veracruz
VASCAN	Database of Vascular Plants of Canada
VIR	Vavilov All-Russian Research Institute of Plant Industry
VNIIMK	All Russian Research Institute of Oil Crops
WGD	Whole genome duplication
WMV	Watermelon mosaic virus
WRPIS	USDA, ARS Western Regional Plant Introduction Station
WTO	World Trade Organization
WUS	Wild utilized species
WY	Wyoming
YUNGA	Youth and United Nations Global Alliance
ZWMV	Zucchini yellow mosaic virus

Part I
Conservation in North America

Chapter 1
Wild Plant Genetic Resources in North America: An Overview

Stephanie L. Greene, Colin K. Khoury, and Karen A. Williams

Abstract North America, including Canada, Mexico, and the United States, is rich in plant species used by humans in both ancient and modern times. A select number of these have become globally important domesticated crops, including maize, beans, cotton, and sunflower. Many other native and also naturalized species have potential for use, either directly or as genetic resources for breeding agricultural crops. However, despite increasing recognition of their potential value, deficiencies in information, conservation, and access to the diversity in these plants hinder their further use. This chapter provides an overview of the agriculturally relevant wild plant resources of North America, with focus on wild relatives of globally important major crops, as well as the wild cousins of regionally and locally important domesticates. The chapter concludes by providing an overview of strategies for conserving wild plant genetic resources, including the international regulatory frameworks affecting policies to various degrees in Canada, Mexico, and the United States.

Keywords Germplasm · Genetic resources · Wild species · Conservation · North America · Crop resources

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S. L. Greene (✉)
USDA, Agricultural Research Service, Center for Agricultural Resources Research, National Laboratory for Genetic Resources Preservation, Fort Collins, CO, USA
e-mail: stephanie.greene@ars.usda.gov

C. K. Khoury
USDA, Agricultural Research Service, Center for Agricultural Resources Research, National Laboratory for Genetic Resources Preservation, Fort Collins, CO, USA

International Center for Tropical Agriculture (CIAT), Cali, Colombia
e-mail: colin.khoury@ars.usda.gov; c.khoury@cgiar.org

K. A. Williams
USDA, Agricultural Research Service, Beltsville Agricultural Research Center, National Germplasm Resources Laboratory, Beltsville, MD, USA
e-mail: karen.williams@ars.usda.gov

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1.1 Introduction

Increasingly variable weather, shifting disease and pest pressures, soil degradation, loss of arable lands, and water scarcity are not only on the horizon; they are already our reality. Canada and the United States are experiencing higher temperatures and more severe weather events, and wildfires (Field et al. 2007). Mexico is undergoing an even greater range of climatic changes, including increased temperatures, especially in the north, decreased rainfall in the central regions, and more storms and prolonged drought during the dry season (National Intelligence Council 2009). Farmers in North America face a turbulent ride as they navigate the Anthropocene to continue to produce a considerable portion of the food, fiber, and other plant-based resources utilized around the world.

An important strategy for preparing for these challenges is breeding plants that can handle the emerging abiotic and biotic challenges. Wild plant species that are closely related to crops are increasingly recognized as some of the most promising genetic resources that plant breeders can turn to in their efforts to develop cultivars adapted to more extreme conditions (Dempewolf and Guarino 2015; Dempewolf et al. 2017; Castañeda-Álvarez et al. 2016; Zhang et al. 2017). They have already proven their worth in breeding (Hajjar and Hodgkin 2007; Maxted et al. 2012). Ironically, however, the very wild species being promoted as essential tools in resolving agricultural problems are themselves vulnerable to the accelerating environmental changes (Jarvis et al. 2008; Lira et al. 2009; Thomas et al. 2016), as well as to the persistent threats presented by habitat modification, pollution, invasive species, and other anthropogenic impacts (Brummitt et al. 2015).

Confounding progress on conservation of these important species is the fact that agriculturally relevant wild plants occupy a niche that is generally neglected by agricultural researchers, who tend to focus their attention on a handful of crops. At the same time, agriculturally relevant wild species are often also overlooked by habitat and endangered species conservation practitioners, who focus on securing rare and threatened taxa and their ecosystems, rather than on safeguarding the intraspecific variation in frequently common, and often weedy, crop wild relatives.

The information in this introductory chapter sets the stage for the rest of the book. We begin by defining essential terms and concepts. We then discuss the process of domestication, focusing on the crops domesticated in North America. We briefly discuss the importance of wild utilized species and then focus on an overview of the occurrence and conservation status of North American crop wild relatives of important crops. We conclude by discussing in broad strokes the general strategies for conserving wild plant genetic resources, including the international regulatory frameworks affecting policies to various degrees in the region.

1.2 An Agricultural Perspective on North America's Wild Flora

The number of native vascular plant species in the three countries increases from north to south. Canada has almost 5860 (Brouillet et al. 2010), and the United States has nearly 16,200 native plant species (Stein 2002). With 23,314 species (Villaseñor 2016), Mexico is a megadiverse country and fourth in the world for number of native vascular plants. Given such broad diversity in species, it is perhaps surprising that modern-day humans use a relatively small number. The United Nations Food and Agriculture Organization (FAO) global aggregate statistics tell us that just 18 or so plants contribute to 90% of the world's calories and that of these, only 2, maize and sunflower, are indigenous to North America (Khoury et al. 2014). However, calories are not the only important component of diet. Plant species also provide protein, fat, starch, fiber, vitamins, minerals, phytonutrients, and flavor. For example, chili peppers, the main cultivated species that originated in North America, are the world's most important spice by essentially all measures. Moreover, many plant species are used for purposes other than food. Examples of important North American species valued for non-food uses include cotton (as a textile), echinacea and American ginseng (as medicines), guayule and jojoba (for industrial uses), hops and vanilla (as flavorings), and rudbeckia and phlox (as ornamentals).

Useful plants fall along a continuum that can be categorized according to the extent that humans have influenced their form. At one end of the continuum are domesticated species, which we call "crops." Crops display a very considerable suite of changes driven by selection pressures placed upon them by humans, typically including the loss of natural dispersal mechanisms; larger sizes of seeds, fruits, or other plant parts; and the loss of dormancy. At the other end are wild species that show no morphological evidence of human use. Both crops and wild species can be managed by humans (e.g., wild fruit trees managed in situ by burning or annual crops cultivated ex situ by planting and harvesting in fields), and management of wild species can lead to domestication (i.e., Casas et al. 2007). The focus of this book is on wild plant species with relevance for agriculture and other human uses, which we term "wild plant genetic resources." These species include the wild plant populations from which domesticated varieties evolved (crop progenitors), wild species that can be used to improve contemporary crops (crop wild relatives), wild species that have a record of use by people (wild utilized species), and any other wild species with potential for future crop development (new crops).

1.2.1 Wild Utilized Species

Historically, wild plant species have underpinned the diets of gatherer-hunter and forager cultures and continue today to contribute significantly to diets, particularly in rural regions of the developing world (Bharucha and Pretty 2010). In Mexico, it is estimated that 5000–7000 wild plant species were used for food and other purposes (Casas et al. 1994; Caballero et al. 1998). North of Mexico, approximately 1800 species have been documented as having been used by the indigenous peoples of North America (Moerman 2003), and Uprety et al. (2012) reported that 546 medicinal plants were used by indigenous peoples in the boreal forests of Canada. Many of these wild food and medicinal species were adopted by early colonists in North America (Turner and von Aderkas 2012), and foraging for wild plant species to use as food or medicine continues to be important in North America. In recent decades, there has been growing interest in using wild plants, especially native species, to revegetate or restore wild lands. For example, in the United States, an alliance of federal and private partners has developed the National Seed Strategy for Rehabilitation and Restoration, driven by federal mandates to use native plant materials (Plant Conservation Alliance 2015).

1.2.2 Domestication in North America

For a select group of plant species, human use has led to domestication. Larson et al. (2014) provide a general definition of domestication as “a selection process for adaptation to human agro-ecological niches and at some point in the process, human preference.” Archeological remains provide ample evidence for the domestication of North American wild progenitors of crop plants, beginning 12,000–8200 BP in Mesoamerica and 8200–4200 BP in temperate regions (Larson et al. 2014). A number of these domesticates have over time become globally important (Table 1.1) (Khoury et al. 2016). A few, such as marsh elder (*Iva annua* L.), little barley (*Hordeum pusillum* Nutt.), and devil’s claw (*Proboscidea parviflora* [Wool.] Wool and Standi), have largely been abandoned (Smith 2006; Bretting 1986).

The process of domestication is driven by the interaction of environmental factors, biology, and human needs, which results in crops that range from plants that differ only slightly from their wild ancestors to species that cannot persist without human interaction (Larson et al. 2014; Meyer et al. 2012). Mesoamerica provides a fine example of this (Lira 2009). Over 20 plant species have been domesticated and have reached globally important food- crop status, including maize (*Zea mays* L.), beans (*Phaseolus* L. spp.), chili pepper (*Capsicum annuum* L.), pumpkins and squashes (*Cucurbita pepo* L.), cotton (*Gossypium hirsutum* L.), avocado (*Persea americana* Mill), cacao (*Theobroma cacao* L.), and vanilla (*Vanilla planifolia* Jacks). Within this same region, a study limited to the Tehuacán-Cuicatlán Valley, Mexico, found that there were over 200 species currently in incipient stages of domestication, the result of management in traditional agricultural systems (Casas et al. 2007; Avendaño et al. 2009; Blancas et al. 2010). Table 1.1 provides a select list of native North American domesticated species, including the approximate time of start of domestication, and the degree to which the crop has changed from its wild counterpart. The list demonstrates that a wide range of crops have been domesticated in North America, that crops have been domesticated from the pre-Columbian

Table 1.1 Selected native North American domesticates

Taxon	Common name	Location	Date[a]	Domestication level[b]	Comments/key references
Agave tequilana Weber and other Agave species	Agave	Yucatan, Mexico	9000	Low	Meyer et al. (2012); Colunga-GarcíaMarín and Zizumbo-Villarreal (2007)
Amaranthus caudatus L., *A. cruentus* L., and *A. hypochondriacus* L.	Amaranth	Mexico	6000	Low	Pickersgill (2007); Janick (2013)
Andropogon gerardii Vitman	Big bluestem	United States	50	Low	Price et al. (2012)
Annona cherimola Mill.	Cherimoya	Southern Mexico	4000	Low	Casas et al. (2007)
Apios Americana Medik.	Indian bean	Midwestern to Northeastern United States	500	Low	Reynolds et al. (1990)
Asimina triloba (L.) Dunal	Pawpaw	Southeastern, United States	100	Low	Meyer et al. (2012)
Bouteloua dactyloides (Nutt.) Columbus	Buffalo grass	United States	50	Low	Riordan and Browning (2003)
Canavalia ensiformis (L.) DC.	Horse bean, jack bean	Mexico	1050	Low	Sauer and Kaplan (1969)
Capsicum annuum L. var. *annuum*	Chili pepper	Highlands of Mexico	6000	High	Meyer et al. (2012)
Carica papaya L.	Papaya	Southern Mexico	2000	Medium	Meyer et al. (2012)
Carya illinoinensis (Wangenh.) K. Koch	Pecan	Southeastern United States	400	Medium	Grauke et al. (2016)
Casimiroa edulis Llave & Lex.	White sapote	Central Mexico	5000	Medium	Meyer et al. (2012)
Chenopodium berlandieri subsp. *jonesianum*	Chenopod	Eastern United States	3700	Low	No longer cultivated/Smith (2006)
Chenopodium berlandieri subsp. *nuttalliae*	Huauzontle	Southern Mexico	700	Low	Smith (2006)
Chenopodium ambrosioides L.	Epazote	Mexico	100	Low	Blanckaert et al. (2012)

(continued)

Table 1.1 (continued)

Taxon	Common name	Location	Date^a	Domestication level^b	Comments/key references
Cucurbita argyrosperma Huber subsp. *argyrosperma*	Silver-seed gourd, green-stripe cushaw	Southwest Mexico	<7000	Medium	Sanjur et al. (2002); Hernandez Bermejo and Leon (1994)
Cucurbita pepo L. subsp. *ovifera*	Squash	Eastern United States	8000	High	Meyer et al. (2012)
Cucurbita pepo L. subsp. *pepo*	Pumpkin	South-Central Mexico	10,000	High	Meyer et al. (2012)
Diospyros nigra (J.F. Gmel.) Perrier	Black Sapote	Mexico	5400	Medium	Meyer et al. (2012)
Fragaria x ananassa Duchesne ex Rozier	Modern cultivated strawberry	Eastern United States	1740	High	*F. virginiana* parent came from eastern North America; however hybrid was developed in France/Meyer et al. (2012)
Echinacea purpurea (L.) Moench	Purple coneflower	Eastern United States	50	Low	Ault (2003)
Gossypium hirsutum L.	Cotton	Eastern Central Mexico	5500	High	Meyer et al. (2012)
Helianthus annuus L.	Sunflower	Eastern United States	4300	High	Meyer et al. (2012)
Helianthus tuberosus L.	Jerusalem artichoke	Eastern United States	1000	Medium	Used by indigenous peoples but major steps in domestication probably by Europeans/ Pickersgill (2007)
Iva annua L. var. *macrocarpa*	Sumpweed, marsh elder	Eastern United States	4000	Low	No longer cultivated/Meyer et al. (2012)
Leucaena spp.	*Leucaena*, guaje	Mexico	3000	Low	Zarate (1999)
Opuntia ficus-indica (L.) mill.	Prickly pear	Central Mexico	9000	Low to medium	Griffith (2004)
Pachyrhizus erosus L. (L.) Urb	Jicama, yam bean	Mexico	4400	Medium	Pickersgill (2007)
Panicum hirticaule J. Presl var. *hirticaule*	Mexican panic grass	Mexico	4000	Low	Nabhan and de Wet (1984)

(continued)

Table 1.1 (continued)

Taxon	Common name	Location	Date[a]	Domestication level[b]	Comments/key references
Panicum virgatum L.	Switchgrass	United States	100	Low	Casler (2012)
Parthenium argentatum Gray	Guayule	United States	50	Low	Ray et al. (2005)
Persea americana Mill.	Avocado	Southern Mexico	7000	Medium to high	Meyer et al. (2012)
Phaseolus acutifolius Gray	Tepary bean	Central or Northern Mexico, Southwestern United States	5000	High	Blair et al. (2012)
Phaseolus coccineus L.	Runner bean	Mexico	900	High	Guerra-García et al. (2017)
Phaseolus lunatus L.	Sieva bean	Central Western Mexico	1800	High	Chacón-Sánchez and Martínez-Castillo (2017)
Phaseolus vulgaris L.	Common bean	Central Mexico	7000	High	Bitocchi et al. (2017)
Phlox paniculata L.	Phlox	United States	50	Low	Zale and Jourdan (2015)
Physalis philadelphica lam.	Tomatillo, husk tomato	Western Mexico	2750	High	Zamora-Tavares et al. (2015)
Podophyllum peltatum L.	Mayapple	United States	50	Low	Lata et al. (2009)
Pouteria sapota (Jacq.) H. E. Moore & Stearn	Mamey sapote	Southeast Mexico	>450	Medium	Arias et al. (2015)
Proboscidea parviflora (Wooton) Wooton & Standl. subsp. *parviflora*	Devil's claw	Southern Arizona, United States, and Northern Sonora, Mexico	1700	Low	Rarely cultivated/ Bretting and Nabhan (1986)
Psidium guajava L.	Guava, guayaba	Southern Mexico	5000	Medium	Ladizinsky (1998); Meyer et al. (2012)
Rubus plicatus Weihe & Ness and hybrids	Blackberry	North American, North of Mexico	150	Medium	Janick (2013)
Rubus occidentalis L.	Black raspberry	North America, North of Mexico	120	Medium	Sauer (1993)
Salvia hispanica L.	Chia	Mexico	450	Medium	Cahill (2005)

(continued)

Table 1.1 (continued)

Taxon	Common name	Location	Date[a]	Domestication level[b]	Comments/key references
Sassafras albidum (Nutt.) Nees	Sassafras	Eastern United States	500	Low	Meyer et al. (2012)
Sechium edule (Jacq.) Sw.	Chayote	Mexico	>450	Medium	Lira Saade (1994)
Setaria parviflora (Poir.) Kerguélen	Knotroot fox tail, bitter grass	Mexico, United States	6000	Low	No longer cultivated/Austin (2006)
Spondias purpurea L.	Jocote, purple mombin, hog plum	Southern Mexico	>450	Low	No archeological evidence; plants widely grown when Europeans arrived/Piperno and Smith (2012)
Spondias mombin L.	Ciruela, hog plum	Southern Mexico	7000	Low	Piperno and Smith (2012)
Theobroma cacao L.	Cacao	Southern Mexico	1500	Medium	Meyer et al. (2012)
Vaccinium corymbosum L.	Blueberry (highbush)	Eastern United States	100	Medium	Meyer et al. (2012)
Vaccinium macrocarpon Ait.	Cranberry	Eastern United States	100	Medium	Meyer et al. (2012)
Vanilla planifolia Jacks	Mexican vanilla	Southeastern Mexico	1800	Low	Lubinsky et al. (2008)
Zea mays L. subsp. *mays*	Corn, maize	Mexico	6250	High	Meyer et al. (2012)
Zizania palustris L.	Wildrice (American)	Northcentral United States	25	Low	Meyer et al. (2012)

[a] First evidence of domestication. Approximate date based on "years ago" (YA) standardized at 1950 as present

[b] Level of domestication: high, cannot survive in the wild; medium, some domestication traits present; low, few domestication traits present

era to the present era, and that the domestication level of the majority of these plants tends to be medium to low.

Domestication causes a number of phenotypic changes, frequently referred to as the domestication syndrome. Pickersgill (2007) discussed morphological changes in New World domesticates, which generally included loss of dispersal mechanisms, increases in size and morphological variation, changes in plant habit, loss of seed dormancy, and loss of chemical and mechanical protection. Changes to developmental and morphological domestication traits tend to occur through selection on transcriptional regulators, while selection on structural genes and regulatory genes influences domestication traits that involve specific metabolic pathways (Doebley et al. 2006; Olsen and Wendel 2013).

1.2.3 Improving Crops Using Wild Genetic Resources

A frequent and unintended result of domestication is a reduction in genetic diversity, the consequence of genetic drift due to limited sampling of only a small subset of individuals from wild populations via the selection of domestication traits (Olsen and Wendel 2013). This process is frequently termed the "domestication bottleneck" (Olsen and Gross 2008). Domestication bottlenecks have been reported in many North American domesticates, such as maize (Wright 2005), common bean (Rendón-Anaya et al. 2017), sunflower (Tang and Knapp 2003), and squash and pumpkin (Kates et al. 2017). Miller and Gross (2011) reviewed the literature for 22 annual and perennial taxa and compared neutral genetic diversity between wild species and their domesticated counterparts. Across the following North American domesticates, the average proportion of total diversity retained in domesticates was 75%: common bean, lima bean, scarlet runner bean, chili pepper, maize, sunflower, pecan, red guaje, columnar cactus, and jocote. Annual domesticates retained an average of 70% of diversity, compared to perennial crops, which had little change (Miller and Gross 2011). Maintenance of variation in perennial crops was attributed to the fact that compared to annual crops, perennials have undergone fewer sexual cycles since domestication as a result of their long juvenile phase, clonal propagation, and a broad range of mating systems.

The diversity studies reviewed by Miller and Gross (2011) highlight that for many crops, potentially useful traits have not only been left behind in their wild counterparts, due to sampling bias, but have also been lost during the selection process because they were not useful traits for de novo domestication (Fig. 1.1). However, with our current efforts to improve crops that are more resilient to climate change, traits left behind in the wild or lost during selection have the potential to provide valuable adaptations to abiotic and biotic stresses, enhance nutritional quality, and improve a host of other attributes (Dempewolf et al. 2017; Zhang et al. 2017).

1.3 Categorizing Wild Genetic Resources by Their Potential for Use in Crop Improvement

We define crop wild relatives (CWR) as "wild plants that can be used to improve crops because they are close enough genetically for successful gene transfer." Harlan and de Wet (1971) developed the "gene pool" concept, based on the relative success of interspecific hybridization, to classify the usefulness of wild taxa for crop improvement. However, these types of studies have not been conducted for all crops, and crossing success may not be indicative of CWR usefulness in crop improvement (Wiersema and León 2016). To counter this, Maxted et al. (2006) proposed the "taxon group" concept, based on infrageneric taxonomic classifications with wild taxa in closer sections or other groupings within the crop genus

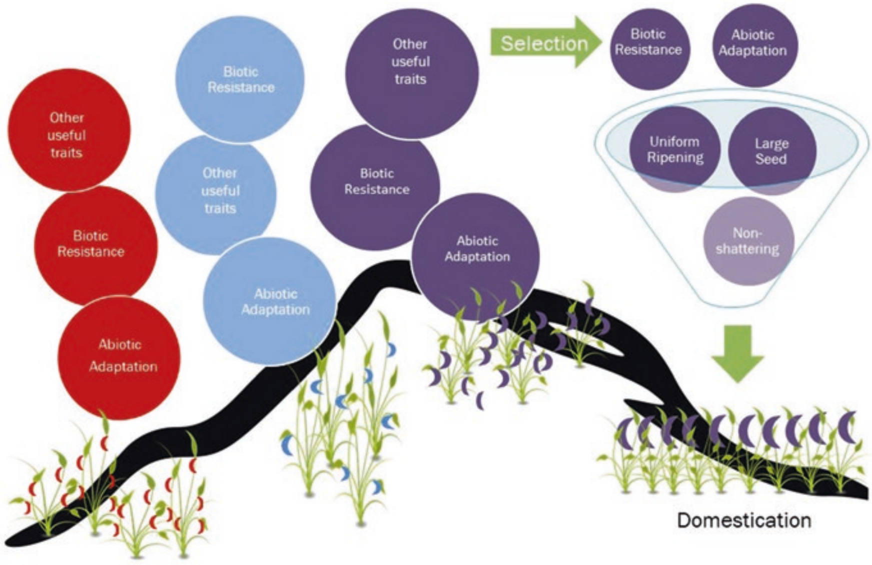

Fig. 1.1 Although the domestication process results in crops more suitable for human use, a general trade-off is the reduction in genetic diversity (relative neutral allelic diversity is represented by the size of bubble). Adaptive traits, the results of natural selection in diverse environments (colors represent ecotypes) may be left behind in wild progenitors due to sampling bias or be lost during selection for domestication traits

being considered more closely related to the crop and thus having higher potential value. However, there are limitations to this approach since some genera lack such classification, and classifications based on morphology may overlook issues such as ploidy differences that influence crossibility. More recently, Wiersema and León (2016) have attempted to integrate the gene pool concept with an enhanced taxon group concept (i.e., phylogenetic and ploidy data are incorporated) to develop genetic relative classes that are descriptive of CWR with crossibility data and predictive of CWR having unknown crossibility with the crop. Primary genetic relatives cross readily with the crop or can be predicted to cross readily due to taxonomic relationships and produce (or can be expected to produce) fertile hybrids with good chromosome pairing. Secondary genetic relatives cross readily or can be predicted to cross readily due to taxonomic relationships but produce (or can be expected to produce) partially or mostly sterile hybrids and have poor chromosome pairing; therefore some effort is needed to overcome barriers to the production of viable offspring. Tertiary genetic relatives can be crossed or can be predicted to cross due to taxonomic relationships but produce (or can be expected to produce) lethal or sterile hybrids, necessitating special techniques (some not yet developed) for successful gene transfer. Wiersema and León (2016) also include a fourth class, “graft stock,” that includes CWR useful as rootstocks or as genetic resources for breeding rootstocks.

Genomic data are rapidly expanding our understanding of the phylogenetic relationships between crops and closely related species and promise to further refine attempts to classify CWR that are based on their usefulness to improve crops. Miller and Khoury discuss this potential further in Chap. 6 (this volume). CWR lists are available from GRIN-Global (https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysearchcwr.aspx) (Wiersema et al. 2012) and from the "Harlan and de Wet Crop Wild Relative Inventory" (https://www.cwrdiversity.org/checklist/) (Vincent et al. 2013).

1.4 Overview of Crop Wild Relatives in North America

An important first step in conservation planning is the creation of a species inventory (Maxted et al. 2012). National inventories of CWR have been published for a growing list of countries, including the United States (Khoury et al. 2013). Mexico is close to publishing an inventory, and Canada has identified this as a priority. At the global level, the Crop Trust (formally known as the Global Crop Diversity Trust, www.croptrust.org) and the Royal Botanic Gardens, Kew, with funding from the Norwegian government, have supported the development of the "Harlan and de Wet Crop Wild Relative Inventory" (Vincent et al. 2013) and a global "gap analysis" of crop wild relatives (Castañeda-Álvarez et al. 2016). The goal has been to develop a global inventory of CWR of major food crops and to gain a better understanding of the representation of these CWR in the world's major public genebanks. The inventory (https://www.cwrdiversity.org/checklist/) contains information on 1667 CWR taxa related to 173 globally important crops (see Vincent et al. 2013 for their definition of globally important crops). These data were used to obtain an overview of CWR found in Canada, Mexico, and the United States. Tables 1.2, 1.3, and 1.4 summarize the number of CWR taxa by genus, taken from the inventory for each country. Canada, with the smallest number of globally important CWR taxa (84), still possesses a large number of taxa closely related to sunflower, currant,

Table 1.2 Number of wild taxa by genus of CWR of internationally important crops, native or naturalized in Canada

Genus	Taxa	Genus	Taxa	Genus	Taxa
Allium L..	2	*Diplotaxis* DC.	2	*Lactuca* L.	4
Armoracia G. Gaertn. B. Mey. & Scherb.	1	*Echinochloa* P. Beauv.	2	*Malus* Mill	2
Barbarea W.T. Aiton	1	*Eruca* Mill.	1	*Prunus* L.	6
Chenopodium L.	3	*Fragaria* L.	11	*Ribes* L.	14
Comarum L.	1	*Helianthus* L.	13	*Rorippa* Scop.	8
Corylus L.	3	*Hordeum* L.	4	*Setaria* P. Beauv.	1
Digitaria Haller	1	*Juglans* L.	1	*Vitis* L.	3

Taken from The Harlan and de Wet Crop Wild Relative Inventory, 2017

Table 1.3 Number of wild taxa by genus of CWR of internationally important crops, native or naturalized in Mexico

Genus	Taxa	Genus	Taxa	Genus	Taxa
Capsicum L.	1	*Ipomoea* L.	8	*Rorippa* Scop.	5
Chenopodium L.	2	*Juglans* L.	11	*Saccharum* L.	2
Cucumis L.	2	*Lactuca* L.	2	*Setaria* P. Beauv.	1
Cucurbita L.	6	*Lupinus* L.	1	*Solanum* L.	24
Digitaria Haller	23	*Manihot* Mill.	6	*Theobroma* L.	1
Echinochloa P. Beauv.	6	*Oryza* L.	2	*Thespesia* Sol. ex Correa	1
Eruca Mill.	1	*Panicum* L.	1	*Tripsacum* L.	17
Fragaria L.	4	*Persea* Mill.	1	*Vasconcellea* A. St.-Hil.	1
Gossypium L.	11	*Phaseolus* L.	50	*Vitis* L.	10
Helianthus L.	9	*Pistacia* L.	2	*Xanthosoma* Schott	6
Hordeum L.	4	*Prunus L.*	4	*Zea* L.	5
Ilex L.	8	*Ribes* L.	2		

Taken from The Harlan and de Wet Crop Wild Relative Inventory, 2017

Table 1.4 Number of wild taxa by genus of CWR of internationally important crops, native or naturalized in the United States

Genus	Taxa	Genus	Taxa	Genus	Taxa
Allium L.	7	*Digitaria* Haller	27	*Panicum* L.	3
Armoracia G. Gaertn. B. Mey. &Scherb	1	*Diplotaxis* DC.	3	*Phaseolus* L.	8
Artocarpus J.R. Forst. & G. Forst.	1	*Echinochloa* P. Beauv.	7	*Pistacia* L.	1
Asparagus L.	4	*Eruca* Mill.	1	*Prunus* L.	23
Avena L.	5	*Fragaria* L.	19	*Pyrus* L.	1
Barbarea W.T. Aiton	3	*Gossypium* L.	2	*Raphanus* L.	3
Beta L.	1	*Helianthus* L.	67	*Ribes* L.	22
Brassica L.	4	*Hordeum* L.	8	*Rorippa* Scop.	23
Capsicum L.	1	*Ilex* L.	7	*Saccharum* L.	7
Carthamus L.	1	*Imperata* Cirillo	1	*Setaria* P. Beauv.	2
Chenopodium L.	5	*Ipomoea* L.	5	*Sinapis* L.	1
Coincya Rouy	1	*Juglans* L.	7	*Solanum* L.	2
Colocasia Schott	1	*Lactuca* L.	6	*Sorghum* Moench	1
Comarum L.	1	*Lathyrus* L.	1	*Thespesia* Sol. ex Correa	1
Corylus L.	4	*Lupinus* L.	1	*Tripsacum* L.	4
Cucumis L.	3	*Malus* Mill.	6	*Vicia* L.	4
Cucurbita L.	2	*Manihot* Mill.	3	*Vitis* L.	25
Daucus L.	2	*Medicago* L.	2		

Taken from The Harlan and de Wet Crop Wild Relative Inventory, 2017 gooseberry, and strawberry. Mexico, with 240 CWR taxa in the inventory, is rich in CWR taxa related to maize and several beans, crops that were domesticated in that region. The United States has 351 CWR taxa (351) listed in this inventory. The large number may be reflective of efforts in the United States to better document native CWR resources. The United States is rich in CWR taxa for sunflower, grape, stone fruits, and small fruits (blackberry, blueberry, cranberry, currant, gooseberry, raspberry, and strawberry) (Table 1.5).

Table 1.5 Native CWR of internationally important crops, which occur in Canada, Mexico, and the United States and which were given a high priority for further collecting based on their limited representation in major ex situ collections, as identified by the Global CWR Gap Analysis project

Canada		
Chenopodium berlandieri Moq	*Helianthus giganteus* L.	*Prunus americana* Marshall
Comarum palustre L.	*Helianthus hirsutus* Raf.	*Prunus emarginata* (Douglas ex Hook.) D. Dietr.
Diplotaxis muralis (L.) DC	*Helianthus maximiliani* Schrad.	*Prunus maritima* Marshall
Diplotaxis tenuifolia (L.) DC	*Helianthus pauciflorus* Nutt.	*Prunus pumila* L.
Fragaria chiloensis (L.) Mill	*Helianthus tuberosus* L.	*Setaria faberi* Herrm.
Fragaria virginiana Duchesne	*Lactuca saligna* L.	*Vitis aestivalis* Michx.
Helianthus divaricatus L.	*Malus fusca* (Raf.) C.K. Schneid.	
Mexico		
Capsicum annuum L.	*Ipomoea ramosissima* (Poiret) Choisy	*Phaseolus longiplacentifer* Freytag
Chenopodium berlandieri Moq.	*Ipomoea tiliacea* (Willdenow) Choisy in D.C.	*Prunus emarginata* (Douglas) Eaton
Cucurbita lundelliana L.H.Bailey	*Lactuca saligna* L.	*Prunus minutiflora* Engelm. ex A. Gray
Cucurbita emall) L.H.Bailey	*Manihot aesculifolia* Pohl	*Setaria faberi* R. A. W. Herrm.
Cucurbita pepo L.	*Manihot angustiloba* (Torr.) Mll. Arg.	*Solanum clarum* Correll
Gossypium harknessii Brandegee	*Manihot chlorosticta* Standl. & Goldman	*Solanum hintonii* Correll
Gossypium turneri Fryxell	*Manihot davisiae* Croizat	*Solanum hjertingii* Hawkes
Helianthus hirsutus Raf.	*Manihot rubricaulis* I.M. Johnst.	*Solanum hougasii* Correll
Helianthus niveus (Benth.) Brandegee	*Manihot walkerae* Croizat	*Theobroma cacao* L.
Ipomoea batatas (L.) Lam.	*Oryza alta* Swallen	*Vasconcellea cauliflora*
Ipomoea cordatotriloba Dennstedt	*Oryza latifolia* Desv.	*Tripsacum dactyloides* (L.) L.
Ipomoea leucantha Jacquin	*Panicum stramineum* Hitchc. & Chase	*Zea diploperennis* H. H. Iltis et al.

(continued)

Table 1.5 (continued)

Ipomoea littoralis Blume	*Phaseolus albescens* McVaugh ex Ramirez-Delgadillo & A. Delgado	*Zea luxurians* (Durieu & Asch.) R. M. Bird
United States		
Allium ampeloprasum L.	*Helianthus debilis* Nutt. *vestitus*	*Panicum nephelophilum* Gaudich.
Artocarpus mariannensis Tr	*Helianthus deserticola* Heiser	*Panicum stramineum* Hitchc. & Chase
Asparagus horridus L.	*Helianthus divaricatus* L.	*Prunus americana* Marshall
Asparagus officinalis L.	*Helianthus giganteus* L.	*Prunus cerasifera* Ehrh.
Avena hybrida Peterm.	*Helianthus maximiliani* Schrad.	*Prunus emarginata* (Douglas) Eaton
Avena trichophylla K. Koch	*Helianthus niveus* (Benth.) Brandegee	*Prunus maritima* Marshall
Capsicum annuum L. *glabriusculum*	*Helianthus niveus* (Benth.) Brandegee *canescens*	*Prunus minutiflora* Engelm. ex A. Gray
Carthamus leucocaulos Sm.	*Helianthus pauciflorus* Nutt. *pauciflorus*	*Prunus pumila* L.
Chenopodium berlandieri Moq.	*Helianthus tuberosus* L.	*Prunus pumila* L. besseyi
Comarum palustre L.	*Ipomoea cordatotriloba* Dennstedt	*Prunus rivularis* Scheele
Cucurbita okeechobeensis (Small) L.H. Bailey subsp. *okeechobeensis*	*Ipomoea leucantha* Jacquin	*Pyrus cordata* Desv.
Daucus carota L.	*Ipomoea littoralis* Blume	*Tripsacum dactyloides* (L.) L.
Daucus carota L. subsp. *gummifer* (Syme) Hook. f.	*Ipomoea tenuissima* Choisy	*Tripsacum dactyloides* (L.) L. var. *dactyloides*
Fragaria chiloensis (L.) Mill.	*Lactuca saligna* L.	*Vitis aestivalis* Michx. *aestivalis*
Fragaria chiloensis (L.) Mill. subsp. *lucida* (E. Vilm. ex Gay) Staudt	*Malus fusca* (Raf.) C.K. Schneid.	*Vitis californica* Benth.
Fragaria chiloensis (L.) Mill. subsp. *pacifica* Staudt	*Manihot angustiloba* (Torr.) Mll.Arg.	*Vitis cinerea* (Engelm.) Engelm. ex Millardet *cinerea*
Fragaria virginiana Mill. subsp. *glauca* (S. Watson) Staudt	*Manihot davisiae* Croizat	Vitis *cinerea* (Engelm.) Engelm. ex Millardet helleri
Fragaria virginiana Mill. subsp. *platypetala* (Rydb.) Staudt	*Manihot walkerae* Croizat	*Vitis labrusca* L.
Fragaria virginiana Mill. subsp. *virginiana*	*Medicago sativa* L. *falcata*	*Vitis monticola* Buckley
Helianthus debilis Nutt. *cucumerifolius* (Torr. & A. Gray) Heiser	*Panicum fauriei* Hitchc.	*Vitis mustangensis* Buckley

Castañeda-Álvarez et al. (2016)

There are many more CWR found in North America than those listed in the Harlan and de Wet Crop Wild Relative Inventory. Although these plants are not genetic resources for globally important food crops, they are important to support the breeding of minor and specialty crops, non-food crops, and for new crop development. Fig. 1.2 illustrates where 618 CWR taxa, including the full set of wild species mapped for the chapters of this book, are concentrated across North America. Regions with a large number of taxa include central and southwestern Mexico, the north and central parts of the eastern United States, the major mountain ranges in Mexico and the United States, and coastal areas. Figures 1.3, 1.4 and 1.5, and 1.6 show the richness of CWR species based on the standard categories of economic plants used in Wiersema and León (2016). Cereals and legumes are concentrated in the central and southern region of western Mexico (Fig. 1.3). Wild genetic resources of vegetables are concentrated in central and southern Mexico, and also in the northern and southeastern United States, as well as the midwestern region of the United States (Fig. 1.4). Wild relatives of fruit crops are concentrated in the northeastern United States and southeastern Canada, as well as in the northwestern United States and southwestern Canada (Fig. 1.5). Wild resources of medicinal, ornamental, industrial, and social use crops are concentrated in the central and eastern United States (Fig. 1.6).

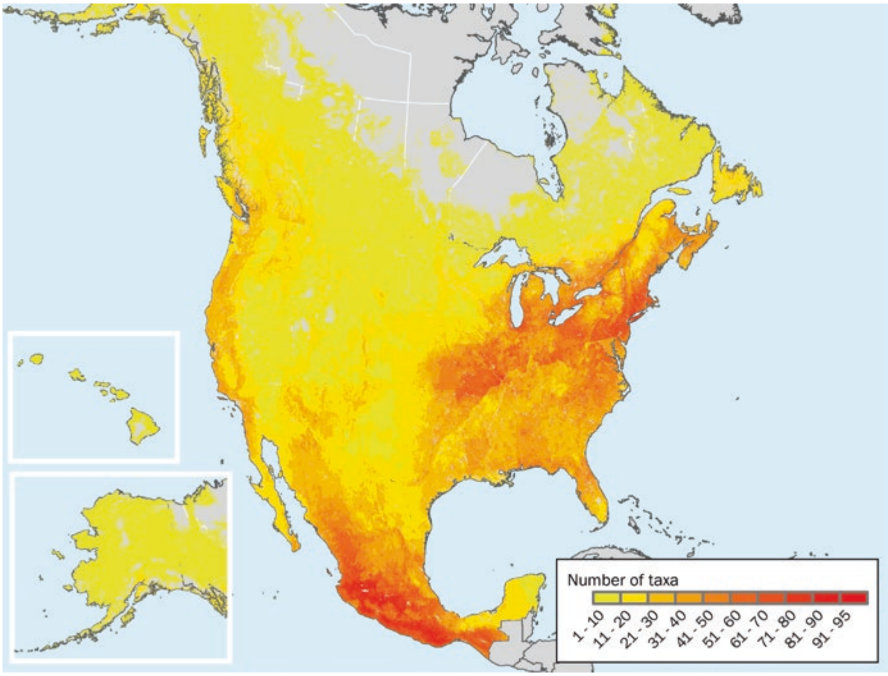

Fig. 1.2 Species richness map illustrating the concentration of crop wild relatives across Canada, Mexico, and the United States. The map displays overlapping potential distribution models for 618 assessed taxa, amounting to all species mapped in this book. Warmer colors indicate areas where greater numbers of taxa potentially occur in the same geographic localities. Full methods for generation of maps and occurrence data providers are listed in Appendix 1

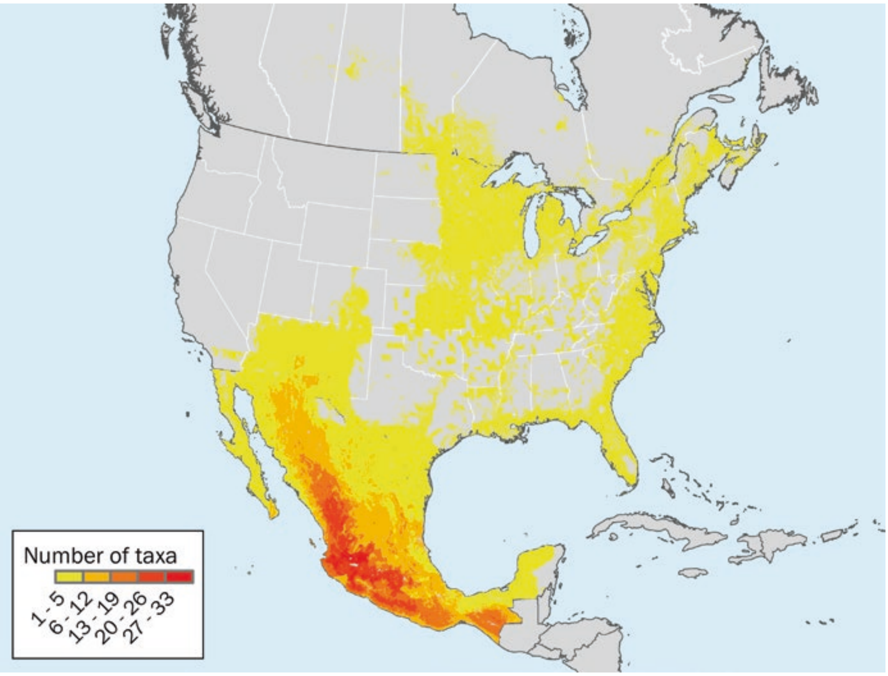

Fig. 1.3 Species richness map illustrating the concentration of 81 crop wild relatives of cereals and legumes mapped in the chapters of this book. Warmer colors indicate areas where greater numbers of taxa potentially occur in the same geographic localities. Full methods for generation of maps and occurrence data providers are listed in Appendix 1

1.5 The Value of North American Crop Wild Relatives

Utilization of CWR in plant breeding has steadily increased over the past decades, providing improved pest and disease resistance, tolerance to abiotic stresses, increased yield, novel cytoplasms, and quality traits (Hajjar and Hodgkin 2007; Maxted et al. 2012; Dempewolf et al. 2017). Advances in breeding, particularly through modern molecular approaches, promise to further facilitate the use of wild germplasm (Zhang et al. 2017; Brozynska et al. 2016; Dempewolf et al. 2017; Prohens et al. 2017).

Well-documented examples exist for the use of North American native CWR. For example, native germplasm was instrumental in developing modern varieties of plum, blueberry, and pecan in the United States (Greene 2012). Perhaps the most important North American CWR utilized since modern breeding began have been the sunflower wild relatives. Dempewolf et al. (2017) identified sunflower as having the most "CWR breeding use" citations among major crops in a recent literature review. The annual economic contribution of sunflower CWR has been estimated between $267 and $384 million USD (Seiler et al. 2017). Most of the value comes

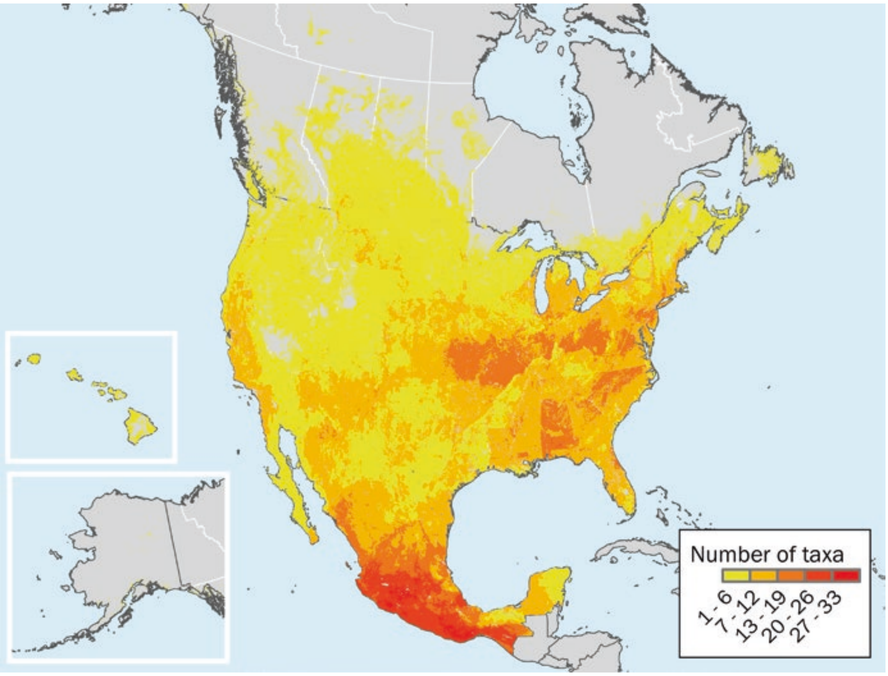

Fig. 1.4 Species richness map illustrating the concentration of 158 crop wild relative species related to, or used as, vegetables, mapped in the chapters of this book. Warmer colors indicate areas where greater numbers of taxa potentially occur in the same geographic localities. Full methods for generation of maps and occurrence data providers are listed in Appendix 1

from the use of the PET1 cytoplasm from *Helianthus petiolaris* (which facilitates the generation of hybrid sunflower varieties), as well as disease resistance genes, abiotic salt tolerance, and resistance to herbicides (Dempewolf et al. 2017).

1.6 Conservation of North American Wild Plant Genetic Resources

An estimated one out of every five plant species worldwide is threatened by habitat loss or modification, agricultural modernization, pollution, over-exploitation, invasive species, and/or climate change (Brummitt et al. 2015). In the United States, 32% of the native flora have been identified as threatened by NatureServe (Havens et al. 2014). Khoury et al. (2013) compiled the conservation status of 3512 taxa in the United States inventory of CWR based on NatureServe rankings. Five taxa were known or presumed extinct in the wild; 4% were ranked as "globally critically imperiled" or "imperiled," and almost 6% were "vulnerable" (see http://explorer.natureserve.org/ranking.htm for definition of rankings). Sixty-two taxa were also

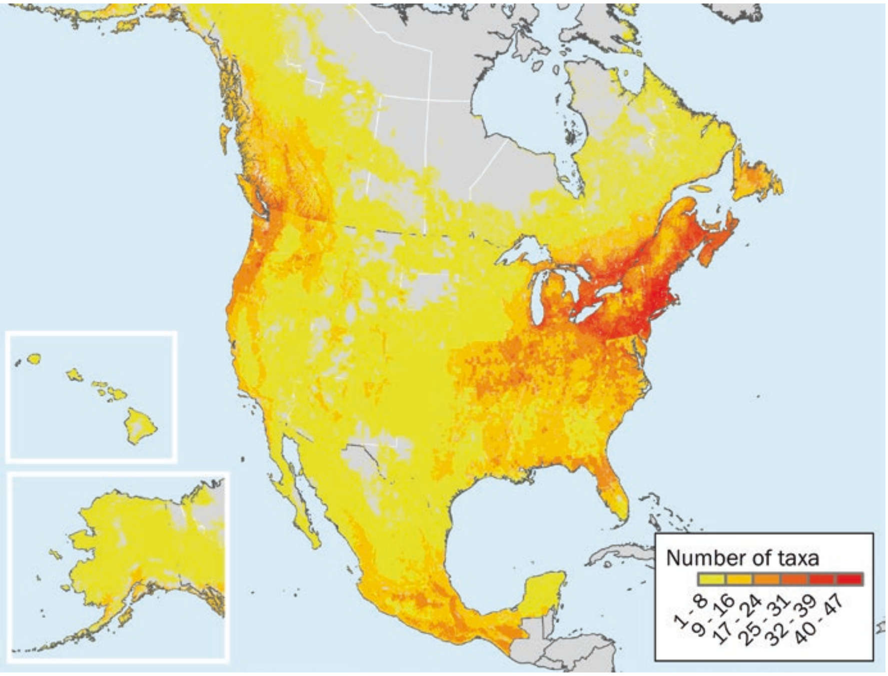

Fig. 1.5 Species richness map illustrating the concentration of 190 crop wild relative species related to, or used as, fruits, mapped in the chapters of this book. Warmer colors indicate areas where greater numbers of taxa potentially occur in the same geographic localities. Full methods for generation of maps and occurrence data providers are listed in Appendix 1

listed as endangered under the Endangered Species Act (7 U.S.C. § 136, 16 U.S.C. § 1531 et seq.). The CWR identified as high priority to collect in the list compiled by Castañeda-Álvarez et al. (2016) included the following North American threatened taxa (based on NatureServe ranking): *Cucurbita okeechobeensis* subsp. *martinezii* (L. H. Bailey) T. C. Andres & Nabhan ex T. W. Walters & D. S. Decker, *Cucurbita okeechobeensis* (Small) L. H. Bailey subsp. *okeechobeensis*, *Fragaria chiloensis* subsp. *sandwicensis* (Decne.) Staudt, *Helianthus niveus* subsp. *tephrodes* (A. Gray) Heiser, and *Manihot walkerae* Croizat.

Threat assessments have also been performed by the International Union for Conservation of Nature (IUCN). From the IUCN Red List for Canada, Mexico, and the United States (IUCN 2017), the following taxa listed in Castañeda-Álvarez et al. (2016) were assigned:

- Near Threatened: *Helianthus exilis* A. Gray.
- Vulnerable: *Helianthus anomalus* S. F. Blake.
- Data Deficient (i.e. insufficient data to make an assessment): *Carica papaya* L., *Cucurbita okeechobeensis* (Small) L. H. Bailey, *Helianthus arizonensis* R.C. Jacks., *H. deserticola* Heiser, *H. niveus* (Benth.) Brandegee, *Prunus havardii* (W. Wight) S. C. Mason, *P. maritima* Marshall, *P. rivularis* Scheele.

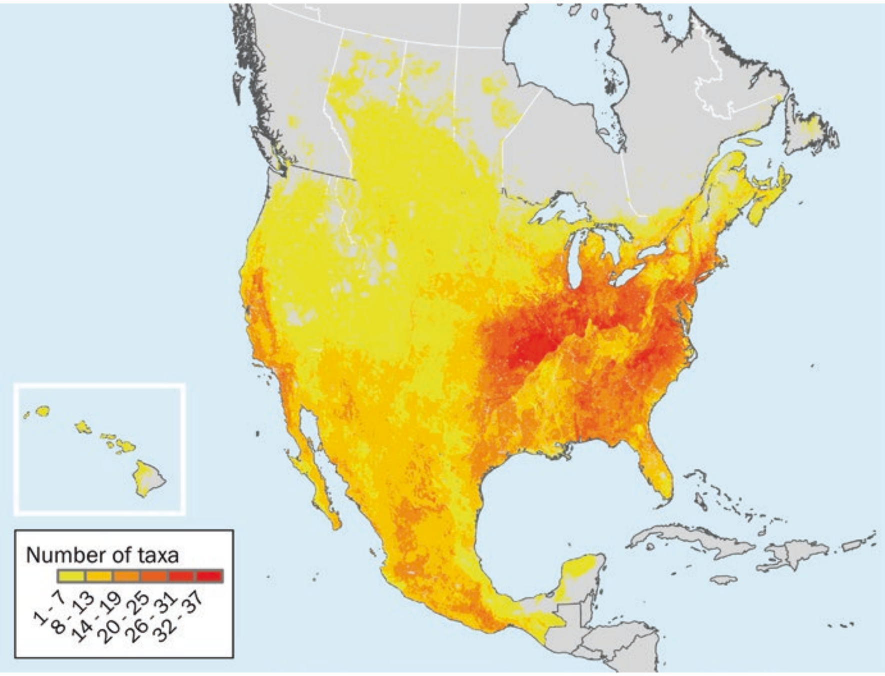

Fig. 1.6 Species richness map illustrating the concentration of 256 crop wild relative species and wild utilized species related to, or used as, medicinal, ornamental, industrial, and social use crops/plants, mapped in the chapters of this book. Warmer colors indicate areas where greater numbers of taxa potentially occur in the same geographic localities. Full methods for generation of maps and occurrence data providers are listed in Appendix 1

The large discrepancy between numbers of threatened species in the United States identified by NatureServe (5935 species) and the IUCN Red List (273 species), pointed out by Havens et al. (2014), underscores the need for more information on species distributions and rarity, especially for wild plant genetic resources, and reflects results based on different methodologies (see Frances et al. (2018) Chap. 7 for description of threat assessment methods).

1.6.1 *Strategies for Conserving Wild Plant Genetic Resources*

The ideal management of wild plant genetic resources (i.e., crop progenitors, crop wild relatives, wild utilized species, and wild species with potential new use) involves a complementary approach incorporating both in situ (conserved "in the wild") and ex situ (conserved outside of the wild in seed or field banks) conservation.

The community of researchers and practitioners conserving and managing biodiversity under in situ conditions has historically viewed ex situ conservation as supplementary (Havens et al. 2014). The common philosophy embraced by this community is reflected by Ralston (2004), who wrote: "A plant is what it is where it is, in situ. In the wild, both the individual plants and the species…are embedded in ecosystems." In contrast, the community that focuses on conserving wild genetic resources for use in crop breeding has prioritized ex situ conservation because access and availability are important considerations. However, there is general and increasing agreement on both sides that the most effective conservation strategies incorporate the strengths of both aspects.

In situ conservation allows the natural trajectory of evolution to occur; plant species continue to coevolve with pests and pathogens and adapt to changing climates. Furthermore, it is sometimes easier to recollect from an in situ population than produce additional wild seed under ex situ conditions (a process called regeneration). It can also be cost-effective to conserve wild genetic resources in situ, especially if many different taxa already occur in a protected area.

On the other hand, ex situ conservation allows rapid access to germplasm needed by the research community. An inevitable limitation of the ex situ strategy for genetic diversity conservation is that it captures a single genetic snapshot, reflecting a wild population's adaptation to the biotic and abiotic conditions when and where it was collected. The degree to which the sample reflects the genetic structure of the original population depends on the adequacy of the sampling. In addition, the assumption that subsequent ex situ seed increases represent the original sample is dependent on minimizing genetic change (through genetic drift or selection) during the regeneration process. The genetic resources community is acutely aware of the challenges of ex situ conservation and attempts to follow protocols outlined in a body of literature guided by the mantra "sample population diversity and maintain genetic integrity" (i.e., Hoban and Schlarbaum 2014; Dulloo et al. 2008; FAO 2014). Although the static nature of ex situ conservation has drawbacks, there is no question that easily accessible ex situ samples provide the means to discover and use valuable diversity and provide backup to in situ populations that may be vulnerable to a myriad of threats. Ex situ accessions have been shown to preserve alleles that were subsequently lost in in situ populations from which they were collected (Greene et al. 2014).

Conservation of wild plant genetic resources requires the cooperation of many players: different federal, state, and tribal agencies, non-governmental organizations including botanical gardens and academic institutes, and, increasingly, citizen scientists and other local groups (Havens et al. 2014). Sometimes the emphasis on making wild genetic resources available for use can conflict with resource management directives aimed at controlling overharvesting or maintaining the genetic integrity of source populations. For example, the US National Plant Germplasm System has been incorporating germplasm from the Bureau of Land Management-led Seeds of Success (SOS) program, which has been collecting wild species in the United States to support restoration activities. Information on SOS accessions is entered in the GRIN-Global database (https://www.ars-grin.gov/npgs), and seed is available to researchers. However, the location where samples were originally collected is not readily available in GRIN-Global due to land managers' concerns that map coordinates will lead to overharvesting of wild populations by unscrupulous collectors. This lack of information has been a stumbling block for companies seeking germplasm adapted to specific areas for the breeding of native species used in restoration. A solution being explored is making accessions searchable by seed transfer zone, which is obtained using the original locations' map coordinates that are themselves not shared. This example illustrates the importance of recognizing that although the *modus operandi* of various partners may not match, open discussion can lead to innovative solutions that meet different organizational missions while moving native plant conservation objectives forward.

1.6.2 International Regulatory Frameworks for Conserving Plant Genetic Resources

The acquisition, distribution, and use of plant genetic resources are regulated at various levels in Canada, Mexico, and the United States. Each country has its own national and state/provincial regulatory frameworks, as do certain indigenous groups within these countries. The specific regulations in all three countries are also influenced by international agreements, particularly the three described in the following sections.

1.6.2.1 The Convention on Biological Diversity (CBD)

The CBD is an international treaty with three main goals: conservation of biodiversity, sustainable use of its components, and fair and equitable sharing of the benefits arising from the utilization of genetic resources. It is the foundation for the current international framework on access and benefit sharing of all biodiversity, including plant genetic resources. The Convention was opened for signature at the United Nations Conference on Environment and Development (the "Earth Summit") in Rio de Janeiro in 1992 and entered into force in December 1993. As of 2017, the CBD has 196 parties, including Canada and Mexico. While the United States signed the Convention in 1993, as of late 2017, it has not ratified the agreement.

1.6.2.2 The Nagoya Protocol

The Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization elaborates on the provisions of the CBD on access to genetic resources and benefit sharing (Moore and Williams 2011). It was adopted by the Conference of the Parties to the CBD in 2010 in Nagoya, Japan, and entered into force on 12 October 2014. The Protocol aims to establish clear requirements and procedures for accessing genetic resources and establishing mutually agreed terms in each contracting party. It obligates contracting parties to provide for the issuance of a permit or its equivalent when access is granted as evidence of the granting of prior informed consent, if required, and the establishment of mutually agreed terms. The benefits to be shared may be monetary, such as royalties, or non-monetary, such as sharing of research results. The Protocol has provisions concerning the traditional knowledge associated with genetic resources held by indigenous and local communities, as well as the rights of these communities to grant access to certain genetic resources.

The Access and Benefit-Sharing Clearing-House (https://absch.cbd.int/) set up by the Protocol shares relevant information, such as on domestic regulatory access and benefit-sharing requirements and on national focal points and national authorities. Parties are to establish checkpoints in their country to collect or receive information regarding whether genetic resources being utilized have been acquired in compliance with relevant laws on access and benefit sharing. The Protocol recognizes that other international instruments addressing access and benefit sharing, such as the International Treaty on Plant Genetic Resources for Food and Agriculture (Plant Treaty), may apply to specific genetic resources and determine the terms in certain cases. Of the three countries covered in this book, only Mexico has ratified the Protocol.

1.6.2.3 International Treaty on Plant Genetic Resources for Food and Agriculture (Plant Treaty)

The Plant Treaty is a legally binding international agreement adopted by the Conference of the Food and Agriculture Organization of the UN in 2001 and entered into force in 2004. Its objectives are the conservation and sustainable use of plant genetic resources for food and agriculture, and the fair and equitable sharing of the benefits arising from their use, for sustainable agriculture and food security. Recognizing that many countries need more straightforward access to agricultural genetic resources occurring outside their borders than is provided by the CBD, the Plant Treaty established a multilateral system (MLS) for access and benefit sharing to facilitate exchange of genetic resources of 64 internationally important crops and forages and (most of) their wild relatives for the purpose of conservation, research, breeding, and training for food and agriculture.

Material in the MLS is transferred on terms specified in a standard material transfer agreement (SMTA) that was adopted by the Governing Body of the Treaty in 2006. The terms prohibit recipients from claiming intellectual property rights on “genetic parts and components, in the form received” that limit access to these resources. The Treaty states that benefits arising from the use of the materials in the MLS should be shared through both non-monetary and monetary mechanisms. Non-monetary mechanisms include the exchange of information, capacity building, and transfer of technology. It also establishes a mechanism for monetary benefit sharing in the form of a benefit-sharing fund. A recipient of germplasm who commercializes a product that incorporates materials from the MLS and is not available for further research and breeding is required to make mandatory payments to the benefit-sharing fund. If the product is available for research and breeding, the payment is voluntary. These funds are to be used to support projects that promote conservation of plant genetic resources, particularly by farmers in developing countries. Canada and the United States are parties to the Plant Treaty.

The three countries thus differ in their participation in the international agreements that most affect access and benefit sharing for plant genetic resources (Table 1.6). The national genebanks in Canada and the United States have placed their public collections into the MLS established by the Plant Treaty, and germplasm is distributed internationally under the terms of the SMTA. A legal framework for international distribution of germplasm from the national collections in Mexico has not yet been established (see Chap. 3).

Access to in situ genetic resources in the three countries depends on the existence of national legislation. The United States is not a party to the CBD and does not require national level collection permits: access requirements are determined by individual landowners or managers, including federal, state, county, and tribal entities. Both Mexico and Canada are parties to the CBD, and thus permission for access is obtained from the designated national authorities to the CBD. Mexico provides national level collecting permits, which are obtained from the Ministry of the Environment and Natural Resources (SEMARNAT). The National Focal Point for the CBD and Nagoya Protocol in Canada is in the National Wildlife Section of the Canadian Wildlife Service, Department of Environment and Climate Change, Canada. There is currently no specific legislation in place in Canada to govern access to genetic resources, although working groups at the federal, provincial, and territorial levels are considering this issue.

1.6.3 Global and Regional Targets and Networks to Conserve Wild Plant Genetic Resources

Growing awareness of the value of crop wild relatives to food security and recognition of the increasing threats to these genetic resources has led to the explicit targeting of the comprehensive conservation of wild relatives by 2020 within the highest-level global agreements on agriculture, development, and conservation, including the United Nations Sustainable Development Goals (SDG). Target 2.5 of

Table 1.6 The status of participation of Canada, Mexico, and the United States in international agreements on access and benefit sharing of plant genetic resources

Country	Party to CBD	Party to Nagoya Protocol	Party to Plant Treaty
Canada	Yes	No	Yes
Mexico	Yes	Yes	No
USA	No	No	Yes

the SDGs states “By 2020 maintain genetic diversity of seeds, cultivated plants, farmed and domesticated animals and their related wild species, including through soundly managed and diversified seed and plant banks at national, regional and international levels, and ensure access to and fair and equitable sharing of benefits arising from the utilization of genetic resources and associated traditional knowledge as internationally agreed.” Similarly, the CBD’s Aichi Biodiversity Target 13 states that “By 2020, the loss of genetic diversity of cultivated plants and farmed and domesticated animals and of wild relatives, including other socio-economically as well as culturally valuable species is maintained and strategies have been developed and implemented for minimizing genetic erosion and safeguarding their genetic diversity.”

The public genetic resources conservation systems in all three North American countries are working on strengthening an already long history of collaboration. Under the umbrella of the Inter-American Institute for Cooperation on Agriculture (IICA; http://www.iica.int/en) North American regional network for agricultural research (PROCINORTE; www.procinorte.net), the NORGEN task force brings together representatives from Canada, Mexico, and the United States to coordinate cooperation and exchange of technical and scientific knowledge in the area of managing and preserving genetic resources. Representatives of the member countries of NORGEN meet yearly and cooperate in activities and knowledge sharing throughout the year. Activities have included participation in development of the 2006 Americas Hemispheric Conservation Strategy for Plant Genetic Resources for Food and Agriculture in the Americas (https://www.croptrust.org/wp-content/uploads/2014/12/AMS_Hemispheric_FINAL_210208.pdf), several workshops on GRIN-Global attended by Mexican and Canadian curators and facilitated by ARS experts, promotion of strategies for in situ conservation by farmers in Mexico, and in vitro and cryopreservation workshop to increase capacity at the Mexico national genebank, and collection and evaluation of germplasm (IICA 2015). A workshop on conservation of ancestral genetic resources was held in Quebec, Canada, in 2016.

1.7 Conclusion

While many of the targets set forth in the international agreements on sustainable development and biodiversity conservation allow for a decade or more to finish the job, conservation of genetic resources is much more urgently prioritized. This is due to the fact that extinction is a permanent and irreversible loss. It may also be because these targets are entirely technically feasible in that given adequate resources and the scientific ability to complete the task already exists. There is no technical reason why North American wild plant genetic resources should be inaccessible to plant breeders and scientists, much less become extinct.

Linkages between the agricultural research and natural resources conservation communities are also growing stronger, giving some hope that these connections will enable the communities to overcome the traditional economic, mandate, and legislative divides between them. The national laws protecting wild species, although currently deficient in their coverage of all vulnerable North American crop wild relatives, do provide a legislative framework for enhancing their conservation. Thus, although we have a long way to go, the essential institutional, policy, and scientific foundations not only exist in North America but also are being actively improved. There is reason to believe that the continent can be successful in its ambitious efforts to comprehensively conserve and make available its wild plant genetic resources. We hope that this book contributes to the foundational knowledge needed to advance this worthy agenda.

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Chapter 2
Genetic Resources of Crop Wild Relatives: A Canadian Perspective

Axel Diederichsen and Michael P. Schellenberg

Abstract Canada is home to about 5087 species of higher plants of which 25% were introduced to Canada either deliberately or by accident. The richness of botanical species is highest in the southern, more densely settled parts of the country. About 364 native Canadian species have direct or potential use in crop development for various usages with particular emphasis on use for berries and as forages. The use of more than 600 plant species by indigenous people for food, medicine, or spiritual reasons is documented, and they practiced agriculture prior to colonization by Europeans with cultivations of corn, garden bean, squash, sunflower, and tobacco. Only a few native species are crop wild relatives of major agricultural crops such as 14 species of the genus *Helianthus* L., which are related to cultivated sunflower. Taxonomists have made major contributions to recognize the potential of the Canadian flora from a utilitarian aspect. Plant breeding in Canada has been done by the government of Canada, by universities, and, more recently, also by the private sector. The focus has been on major crops, while the activities in forages and minor crops have decreased. Canada maintains a national genebank for ex situ conservation (Plant Gene Resources of Canada). More activities to enhance the complementarity between in situ and ex situ conservation should be undertaken in Canada. The potential of native rangeland plants or weeds as genetic resources is emphasized. The "integrating genebank" is suggested as an ambitious concept. In order to maintain the future options Canadian biodiversity holds for enhancing life and livelihoods, a national strategy is required.

Keywords Native Canadian plant species · Ex situ conservation · In situ conservation · Weeds · Indigenous food plants · Plant genetic resources · Taxonomy · Diversification of agriculture · Native rangeland plants · Nutrition · National biodiversity strategy · Invasive species · Wild fruits

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A. Diederichsen (✉)
Agriculture and Agri-Food Canada, Plant Gene Resources of Canada, Saskatoon, SK, Canada
e-mail: axel.diederichsen@agr.gc.ca

M. P. Schellenberg
Agriculture and Agri-Food Canada, Swift Current Research and Development Centre,
Swift Current, SK, Canada
e-mail: mike.schellenberg@agr.gc.ca

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2.1 Broad Overview

Canada's land area amounts to 9,984,670 km^2 which is about 7% of the terrestrial surface of the globe. The longest east-west distance is about 5514 km, and the longest north-south distance is 4634 km. The arctic tundra in the north is vast, and the boreal forest stretches as a belt from the Pacific to the Atlantic coast. These two vegetation zones cover two-thirds of the country and are very sparsely settled. Canada has 15 terrestrial ecozones that are subdivided into 53 ecoprovinces with 194 ecoregions. Figure 2.1 shows the large forested band that covers more than 40% of Canada's land surface and includes, from west to east, the Pacific Maritime, Boreal Cordillera, Montane Cordillera, Boreal Plains, Boreal Shield, Hudson Plains, and Atlantic Maritime ecozones.

Politically, Canada is divided into ten provinces and three territories. Only the southern parts of the country are densely settled and conducive for agricultural plant

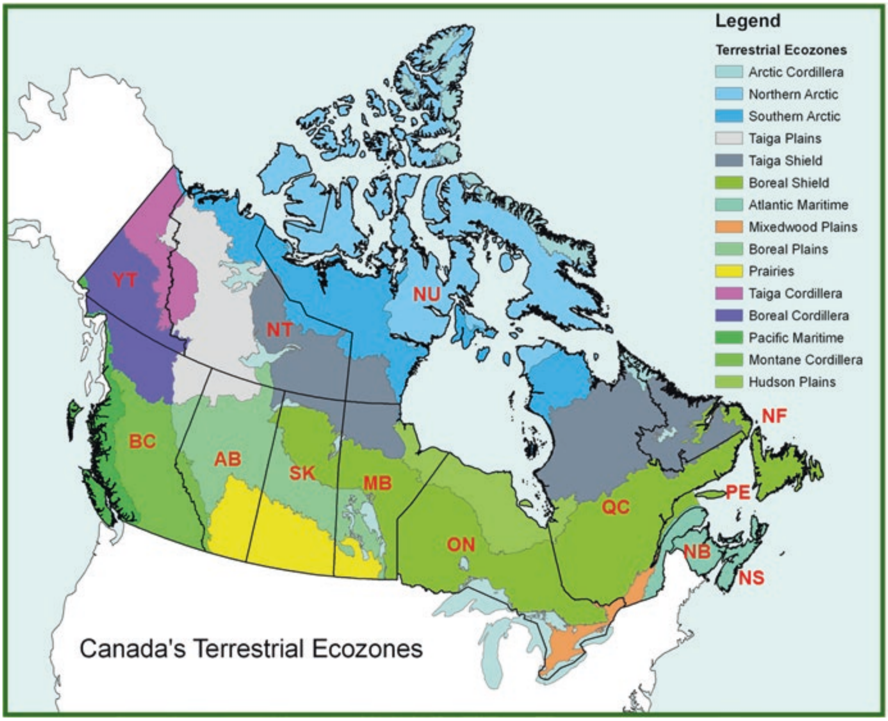

Fig. 2.1 Canada's terrestrial ecozones with borders of provinces and territories indicated (Natural Resources Canada 2017). Abbreviations: AB Alberta, BC British Columbia, MB Manitoba, NB New Brunswick, NF Newfoundland and Labrador, NS Nova Scotia, NT Northwest Territories, NU Nunavut (territory), ON Ontario, PE Prince Edward Island, QC Québec, SK Saskatchewan, YT Yukon (territory)

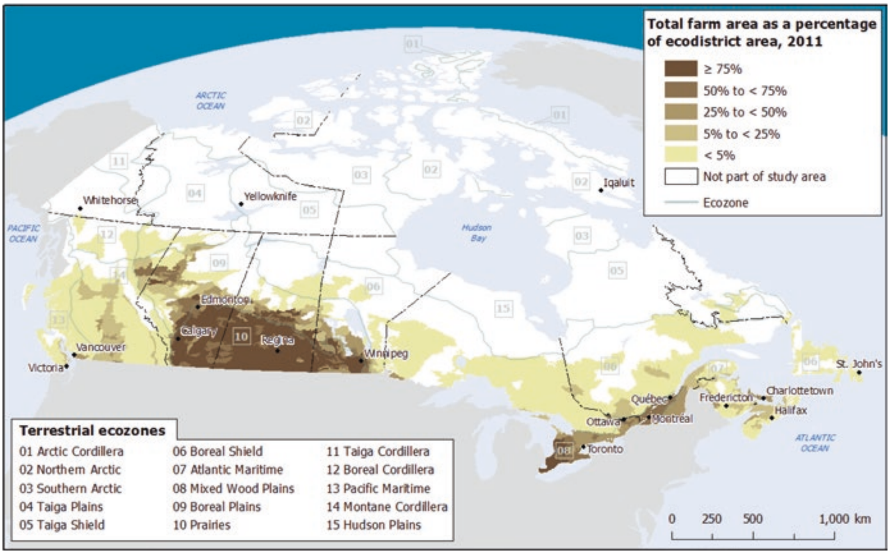

Fig. 2.2 Farmland distribution in Canada. (Statistics Canada 2014)

production. These areas also have more diversified vegetation than the vast northern regions. For agriculture, the prairies of Western Canada are most important (Fig. 2.2). Canada was nearly completely covered by inland ice during the last ice age. As a result, there are only a few pockets with species of preglacial origin, and the number of species endemic to Canada is low.

The number of species of higher plants reported for Canada is 5087 (Candian Food Inspection Agency 2008). Of these, 10 plant species are categorized as being endangered, 47 as threatened, and 46 as being of special concern (COSEWIC 2017). It is remarkable that 1229 vascular plant species, which amounts to about 25% of the total Canadian flora, consist of alien species that were introduced to Canada (Canadian Food Inspection Agency 2008). Of the alien species, 483 are weedy or invasive, and more than half of these were introduced deliberately as crops, as ornamentals, or for other usages. Darbyshire (2003) provided an inventory of Canadian weeds listing 872 species in total, including the native weed species. Due to the size and ecological diversity of the country, 316 native Canadian species are considered introduced in some parts of the country, and of these 69 are categorized as invasive. The degree of impact anthropogenic activity has had on the Canadian flora is tremendous. In the agricultural areas of the western provinces of Alberta, Saskatchewan, and Manitoba, there are only relicts of native habitats left. Figure 2.3 shows that the number of invasive species is particularly high in the more densely settled areas in the southern parts. Although alien species can be invasive and therewith threaten the native flora, it is important to remember that many alien species are crops and crop wild relatives or have potential for use. Trade and colonization have brought many

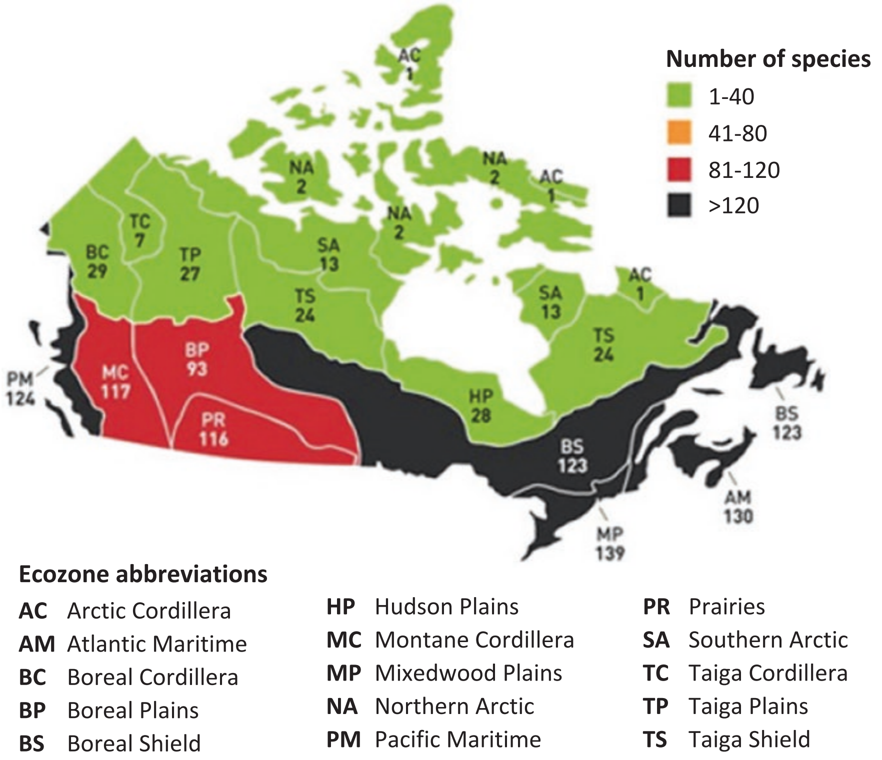

Fig. 2.3 Number of invasive alien plant species in Canada by ecozone. Based on 162 species for which distribution maps were available. (Canadian Food Inspection Agency 2008)

species from Europe, China, and Japan. Figure 2.3 is an approximation, as many of the less populated areas in Canada have no detailed floristic inventories. An excellent and steadily updated checklist for Canadian vascular plants with distribution maps is available on the Internet (Brouillet et al. 2010).

Davidson (1995) reported that 364 native Canadian species have direct or potential use in crop development or breeding programs. He grouped these plant genetic resources into the following categories: forage and turf grasses (138 species); fruit crops (111 species); cereals, oilseeds, and other field crops (18 species); special and minor crops (86 species); and nut crops (11 species). In addition, Davidson (1995) listed 137 genera of native landscape plants and concluded that the potential of these remains untapped. That such a high number of native Canadian taxa are plant genetic resources of economic significance is striking, as Canada is not located in one of the centers of diversity of cultivated plants first pointed out by Vavilov (1926). Nearly all important crops presently grown in Canada trace their evolutionary origin to other parts of the world.

The agriculture of the indigenous people of Canada that existed long before immigration of Europeans cultivated a few plants, namely, maize (*Zea mays* L.), garden bean (*Phaseolus vulgaris* L.), squash (*Cucurbita pepo* L.), Jerusalem artichoke (*Helianthus tuberosus* L.), tobacco (*Nicotiana rustica* L.), and possibly sunflower (*Helianthus annuus* L.). These plants also have their evolutionary origin outside of Canada in the more southern parts of the North American continent. Relict cultivation of these plants using autochthonous (native) genetic resources may still exist in the province of Québec (Gros-Louis and Gariépy 2013). Some of the diversity of these crops has been preserved due to conservation in the national genebanks of Canada and the United States (PGRC 2016). Wildrice (*Zizania palustris* L., *Z. aquatica* L.) is a special case. It has been used by indigenous people long before colonization, but it is not a cultivated plant species as such, although some habitat management may justify it being considered partly domesticated, a status it may share with *Helianthus tuberosus*. There is insufficient knowledge about the interactions between plants and man prior to immigration by Europeans. Kuhnlein and Turner (1991) presented a monograph on this subject. Exploring these questions with focus on livelihoods and future directions recently gained some momentum (Agriculture and Agri-Food Canada 2017) and is also emphasized more on the international scale (Heywood 1999a).

None of the crop wild relatives found in Canada belong to the primary genepool of a major agricultural field crop. Regarding the secondary and tertiary genepool for sunflower breeding, there are 14 *Helianthus* species in Canada that are considered crop wild relatives (Brouillet et al. 2010; Kantar et al. 2015). However, many berries and fruits have wild relatives in Canada that belong to the primary genepool for breeding and were domesticated here. Canada has many crop wild relatives that belong botanically to the same genera as cultivated plant species and thus represent crop wild relatives or genetic resources of the secondary and tertiary genepools for important crops according to the genepool concept of Harlan and De Wet (1971). Catling and Cayouette (1994) emphasized that in particular in small fruits, berries, and nuts, unique endemic genetic resources occur in the Gaspé Peninsula of Québec.

Catling and Porebski (1998) added to the understanding of these native Canadian plant genetic resources by identifying 56 taxa of rare wild plants that are the highest priority for protection on the basis of both rarity and economic value and pointed out that 60% of these taxa occur in the Carolinian zone, which includes the Lake Erie lowland region in Ontario, which is densely populated and in which a decline in crop wild relative populations has been reported by these authors. The map provided in Fig. 2.4 singles this area out as being potentially rich in crop wild relatives.

One should note that the plant taxa found in endangered grasslands of Canada have largely been unexplored for their potential economic impact. Figure 2.4 demonstrates, based on 39 crop species, that the areas of the highest potential concentration of crop wild relative species are also the most densely settled areas of Canada, and hence the areas most threatened by genetic erosion. At the same time, these areas have the highest number of alien species and alien invasive species (compare Fig. 2.3) competing with native species. Figure 2.4 is an approximation, as only 39

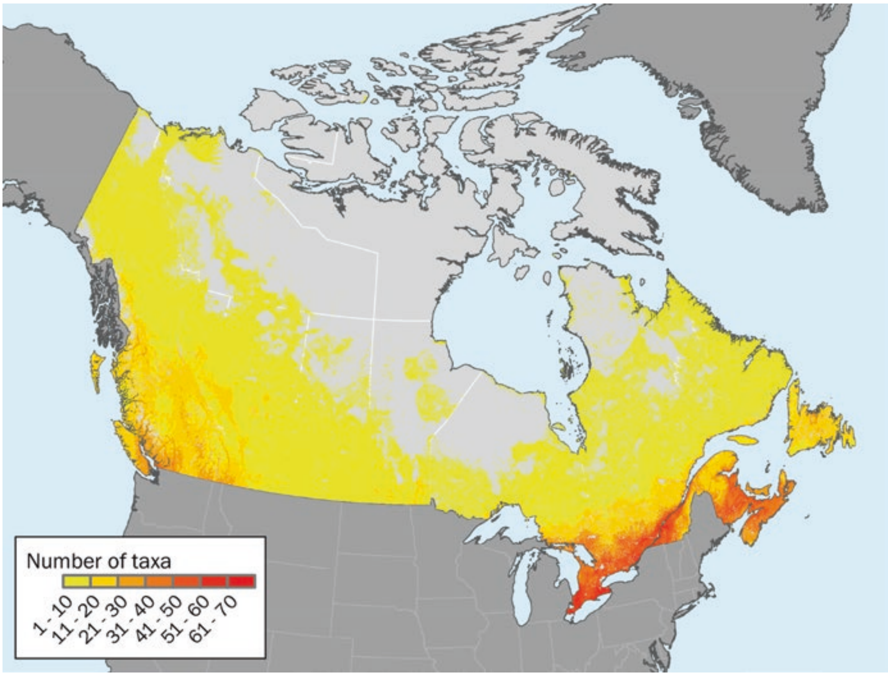

Fig. 2.4 Estimated potential richness of taxa in Canada that are crop wild relatives based on 107 species and 16 genera using data of climatic and edaphic similarities of the assessed area with herbarium and genebank reference localities. Richness of potential distributions of crop wild relative taxa increases from yellow to red. The method for generating the map and a list of reference data providers is given in Appendix 1. Based on online database: Crop Wild Relatives and Climate Change (2013)

crop species were considered and only the data of collection holdings in genebanks and herbaria collections that have been digitized was used to generate this map.

Despite being less rich in species and intraspecific diversity, the arctic regions of Canada also deserve attention as reservoirs of plants with special adaptation, in particular grasses and sedges (Small and Cayouette 2016). The arctic and subarctic ecosystems are very fragile and massively impacted by climate change (Richards 2006).

Many native Canadian plant species, despite not being wild relatives of important cultivated plant species, have great potential for food and agriculture. Indigenous people have used many of these prior to European immigration, and cultivation of some of them is more recent. Marles et al. (2000) compiled information on more than 200 species in Canada's northwest boreal forest used by indigenous people for nutrition or as sources of functional foods, nutraceuticals, or medicines. Arnason et al. (1981) listed 175 species for food, 52 for beverages, and 400 for medicinal use by indigenous people in Eastern Canada. The majority of these species are native to Canada. The fruit crops have become of particular economic relevance, but their potential has not yet been fully developed (St-Pierre 1992; Catling and Cayouette

1994). Among these, blueberry (*Vaccinium myrtilloides* L.) and cranberry (*Vaccinium macrocarpon* Aiton*),* as well as related *Vaccinium* species, have been the basis for increased production and are an important contributor to farm income in Canada. In Western Canada, the introduction of blue honeysuckle (*Lonicera caerulea* L.) as a new crop is an example of successful exploration of native and foreign genetic resources, and collections were made in Canada (Bors 2009). Juneberry (*Amelanchier alnifolia* Nutt.), also known as saskatoon berry, is another fruit in which Canada is a world resource for unique native genetic diversity (Catling and Cayouette 1994). Small (2014) has recently provided a compendium of 100 species of indigenous food plants from North America covering the area of the United States, Mexico, and Canada with emphasis on species of greatest economic potential. Among the many berries and medicinal plants that were used by the indigenous people of Canada, there are rare species that have not been explored for cultivation (Turner 1981). Cultivation may be a solution to avoid them being over-collected from the wild due to increased interest in natural medicines and natural foods.

Despite the mentioned efforts, agricultural research and public breeding in Canada has during the last two decades narrowed its focus to major crops. Research on native species has languished resulting in the potential of many native species being unrealized. Even in the important native berry and fruit species, the public sector and universities have reduced their activities and breeding of these crops. Three factors are largely responsible for neglecting the potential of native Canadian plant diversity for cultivation. Firstly, European immigration changed land-use patterns drastically by establishing agriculture in the areas suitable for that activity. This resulted in agroecosystems replacing natural ecosystems with major changes in species composition and also causing loss of native plant diversity that had potential for cultivation. Secondly, the major influx of immigrants, starting in the middle of the nineteenth century, made it necessary for the Canadian government to support these immigrants immediately and effectively by introducing familiar crops from the Old World that had proven yield potential but needed to be adapted to the local conditions of different regions of Canada. This was essential for national food security in Canada at that time and has been the priority ever since, as Canada became a main exporter of agricultural products during the twentieth century. Cereal research dominated until the middle of the twentieth century, while research in rapeseed and pulse crops is much more recent. Thirdly, much indigenous knowledge about the usages and potential of native Canadian plants has been lost due to the Eurocentric scientific approach that has been dominating the last centuries, and only recently has science started to recognize the relevance of indigenous knowledge.

Canadian Confederation in 1867 and the Experimental Farm Station Act from 1886 were the basis for forming the Research Branch of the Department of Agriculture for Canada in 1886 (Anstey 1986), which today is the Science and Technology Branch of the Department of Agriculture and Agri-Food Canada (AAFC). During the late nineteenth and early twentieth centuries, Canada actively invited immigrants to settle the arable area that now forms part of the three Prairie Provinces, Manitoba, Saskatchewan, and Alberta, which became the most important areas for agricultural production in Canada (Fig. 2.2). The Central Experimental

Farm in Ottawa, Ontario, distributed seed samples of improved crops to farmers. In 1889, 3700 packages of seeds were distributed mostly to farmers (Anstey 1986). These seed packages were larger than the 100 seeds per sample a modern genebank ships to its clients, which are today mostly plant breeders and other researchers. Parenthetically, the Canadian genebank Plant Gene Resources of Canada (PGRC) today also ships about 4000 seed samples annually. Dominated by European immigration and mindset, research on utilization in agriculture rarely focused on native plant species for potential cultivation. As a consequence, large ex situ research collections that entered the Canadian national genebank, PGRC, were established for the crops and crop wild relatives of the cereals oat, barley, and wheat, all of non-Canadian origin. Even within these cereals, the native Canadian taxa were neglected. Similarly, native Canadian grasses, legumes, and other native Canadian forage species have not received much attention. Indigenous people have utilized the medicinal properties of native Canadian species for centuries, and some records exist of their usage by early immigrants. However, over time synthetic drugs have gained much more attention from research, and only toward the end of the twentieth century, the efficacy of several native species as herbal medicines and food sources has become more widely recognized (Arnason et al. 1981; Turner 1981 and Marles et al. 2000). European explorers of Canada that arrived following John Cabot's landing on the Canadian Atlantic coast in 1497 definitely had the exploitation of resources in mind, but the agricultural use and the wild plant use by the indigenous people was not well-respected and only poorly documented (Asselin et al. 2014, 2015, 2017). We are still in the initial stages of realizing the potential of native Canadian plant genetic resources for food and agriculture, medicinal usage, or other purposes (Small 1999). We have lost indigenous Canadian knowledge and know-how, but new knowledge and know-how has also been brought and generated by the European settlers when adapting to the Canadian environment. In addition, indigenous people have also started to use and learn about the new plants that came to Canada.

2.2 Plant Genetic Resources Conservation and Use Policies of Canada in the International Context

Canada was among the first countries to sign the Convention on Biological Diversity (CBD) agreed upon in Rio de Janeiro in 1992 and ratified it the same year (Harvey and Fraleigh 1995). The secretariat of the CBD is located in Montreal, Québec, underlining the high status of this agreement in Canada. Following the CBD ratification, Canada formulated a Canadian Biodiversity Strategy (Minister of Supply and Services Canada 1995). The Canadian Department of Agriculture (Agriculture and Agri-Food Canada, AAFC) published inventories of industry and departmental initiatives related to the Canadian Biodiversity Strategy (Agriculture and Agri-Food Canada 1997a, 1997b). Provinces such as Québec also responded by developing an implementation strategy and an action plan (Gouvernement du Québec 1996a, 1996b). In 2004, the Canadian Council of Ministers of the Environment created the "federal, provincial, and territorial working group on biodiversity", which formulated the "2020 Biodiversity Goals and Targets for Canada" and a Canadian Biodiversity Strategy (Federal, provincial and territorial working group on biodiversity 2016). Canada presented four goals covering 19 targets that relate to the 20 Aichi Biodiversity Targets of the CBD (CBD 2016a). Canada has developed a Federal Sustainable Development Strategy, and environmental indicators have been established (Environment and Climate Change Canada 2016a). Besides the protection of species at risk, these targets also address biodiversity at the landscape level, including agricultural working landscapes. The Canadian federal, provincial, and territorial working group on biodiversity initiated the further development of domestic access and benefit-sharing policy in Canada that also addresses aspects of traditional knowledge.

Finalization of this Canadian policy is very close. The Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization (ABS) of the Convention on Biological Diversity, adopted at the tenth meeting of the Conference of the Parties in Nagoya, Japan on 29 October 2010, has not yet been signed by Canada, and, therefore, it is not legally binding on Canada. The Nagoya Protocol entered into force on 12 October 2014. Presently, Canada is considering becoming a party to the Nagoya Protocol, but a final decision is still pending. The national lead for the CBD and Nagoya Protocol is the Department of Environment and Climate Change Canada.

The International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), adopted in 2001 and signed and ratified by Canada on 10 June 2002, focuses on plant genetic resources for food and agriculture, including crop wild relatives. Canada has been actively involved in negotiating this Treaty, and the Canadian Department of Agriculture is the national lead in negotiations and implementation of the ITPGRFA (Fraleigh and Harvey 2011). The ITPGRFA entered into force on 29 June 2004. Canada is very aware of the international interdependencies for global and national food security, and of the fact that the international exchange of genetic resources for cultivated plants is critical for sustainability both nationally and globally. Canada is particularly dependent on access to genetic resources internationally because most important agricultural crops have their origin outside of the country. Canada also supports the fair and equitable sharing of benefits arising from the use of plant genetic resources which motivates the strong engagement of Canada in the ITPGRFA. Canada recognizes the ITPGRFA as the basis for all plant genetic resources for food and agriculture and made all its genetic resources holdings at the national genebank, Plant Gene Resources of Canada (PGRC), available nationally and internationally under the provisions of the Standard Material Transfer Agreement of the ITPGRFA. This also includes the native Canadian germplasm held by PGRC.

Within Canada, national legislation impacts the diversity of cultivated plants, weeds, and crop wild relatives. The regulatory agency for implementing these laws is the Canadian Food Inspection Agency (CFIA). The Plant Protection Act of 1990 prohibits the import or spread of pests or any other agents that may cause harm to agricultural or forestry plants in Canada (Minister of Justice 2016a). The Seeds Act of 1985 refers to the registration of varieties of cultivated plants but also seed trade and movement of seeds (Minister of Justice 2016b). As such, the Seeds Act includes a regulation with a list of species considered as weeds based on the potential harm they may cause to Canadian agriculture and the environment. The list includes crop wild relatives such as *Aegilops cylindrica* Host, *Elymus repens* (L.) Gould, *Datura stramonium* L., *Raphanus raphanistrum* L., *Avena fatua* L., *A. sterilis* L., *Pastinaca sativa* L., and *Daucus carota* L. In addition, many Canadian provinces have a Weed Control Act with lists of plants in categories according to the degree of harm they may cause in the region. In 2004, Environment Canada (the name of the department changed to Environment and Climate Change Canada in 2015) developed an invasive alien species strategy for Canada in which it clearly expressed the lack of taxonomic benchmarks and expertise that is critical to act on these issues (Environment and Climate Change Canada 2016b). Strong concerns regarding the taxonomic expertise in Canada were articulated by Small et al. (1995): “Little wonder there is increasing doubt that Canada can adequately protect its own biodiversity.” More recently, the Council of Canadian Academics (2010) also expressed concerns in this regard.

The Species at Risk Act from 2002 (Minister of Justice 2016c) is administered by the Canadian Endangered Species Conservation Council, consisting of the Minister of Environment and Climate Change, the Minister of Fisheries and Oceans, the Minister responsible for the Parks Canada Agency, and provincial or territorial ministers responsible for the conservation and management of wildlife species in that province or territory. The Species at Risk Act (Minister of Justice 2016c) was created to be compliant with the CBD, and its main purpose is to protect wildlife species from being extirpated or becoming extinct and to help reestablish lost species. It includes lists of organisms of all kingdoms by species categorized as being extinct, extirpated, endangered, threatened, or of special concern. These lists are updated regularly by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) and can be inspected on the Internet (COSEWIC 2017).

Canadian policies relevant for the use of plant genetic resources for food and agriculture include the Canadian Plant Breeders’ Rights Act from 1990, which was updated in 2015. It now includes provisions that bring it into line with the 1991 amendment of the Convention of the International Union for the Protection of New Varieties of Plants (Minister of Justice 2016d; UPOV 2016).

Canada has been involved in the CBD from the very beginning. Individuals that act as Canadian National Focal Points for several topics including one for the Nagoya Protocol have been nominated (CBD 2016b). Additionally, Canada is actively engaged in the work of the Food and Agriculture Organization (FAO) of the United Nations Commission on Genetic Resources as well as in the ITPGRFA and has National Focal Points for each of them (FAO 2016a, 2016b). This was of particular relevance as Canada had been the only partner country from the North American region that had ratified the ITPGRFA until September 2016, when the United States joined the ITPGRFA. Several Canadian provinces have developed biodiversity strategies in line with targets from the CBD. On the provincial level, biodiversity related to food and agriculture receives less attention than biodiversity of wild and native species.

2.3 Ex Situ Conservation

2.3.1 National Germplasm Collections

Three research centers of Agriculture and Agri-Food Canada share the responsibility for active national genebank work in plant genetic resources for food and agriculture: (1) Plant Gene Resources of Canada (PGRC) at the Saskatoon Research and Development Centre, Saskatoon, Saskatchewan, preserves all seed germplasm, has the central seed storage vaults, and maintains the national genebank information system (GRIN-CA/GRIN-Global-CA) that allows national and international clients and the public to inspect and access Canadian genebank holdings (PGRC 2016); (2) the Canadian Clonal Genebank (CCGB) at the Harrow Research and Development Centre, Harrow, Ontario, preserves fruit germplasm; and (3) the Canadian Potato Genetic Resources (CPGR) at the Fredericton Research and Development Centre, Fredericton, New Brunswick, preserves potato germplasm. The mandate of PGRC is to acquire, preserve, evaluate, and make available the genetic diversity of cultivated plants and their wild relatives with emphasis on germplasm of economic importance or potential for Canada. The germplasm holdings of PGRC and the associated locations currently include 110,442 accessions covering 47 botanical families, 258 genera, and 980 botanical species (Fig. 2.5). Passport, characterization, and evaluation data are accessible via the Internet (PGRC 2016). The cereals, barley, oat, and wheat account for more than 80% of all germplasm holdings. Extensive collections of crop wild relatives exist at PGRC for barley, oat, and wheat, which are important to Canada (Table 2.1). In these groups the proportion represented by crop wild relatives amounts to 30% of the PGRC collection due to intensive collection with Canadian participation of such material in the second half of the twentieth century. Although these wild and weedy crop wild relatives are mostly not

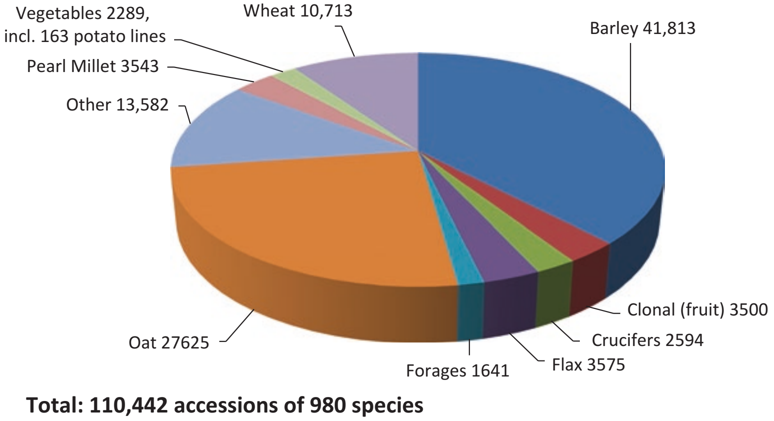

Fig. 2.5 Germplasm holdings at Plant Gene Resources of Canada. (Number of accessions)

Table 2.1 Number of cultivated and wild taxa and accessions of cereals at Plant Gene Resources of Canada (PGRC)

Genus	Total	Cultivated		Wild		Proportion
	Accessions	Taxa	Accessions	Taxa	Accessions	Wild accessions (%)
Aegilops L.	632	–	–	22	632	100
Avena L.	27,790	6	12,502	25	1,5288	55
Hordeum L.	41,813	2	38,858	38	5955	14
Triticum L.	11,345	21	8357	4	2988	26
Total	81,580	51	59,717	67	24,863	30

of Canadian origin, they are important from a national and global perspective, as many were collected in the 1970s to 1990s in regions of the world that are today not easily accessible for such collecting (Baum et al. 1972). Utilization of crop wild relatives in disease resistance breeding has been particularly strong in *Avena* L. (Diederichsen 2016) but also in the genera *Triticum* L., *Aegilops* L., and *Secale* L. By preserving and providing access to these genetic resources, Canada makes significant contributions to the global efforts to conserve and utilize crop wild relatives.

GRC's main germplasm holdings include many accessions for which the GRIN-CA database lists the country of origin as Canada and the accession status as wild. These native Canadian plants include many species that are used as forages (e.g., purple prairie clover, *Dalea purpurea* Vent., *Medicago* L. spp., other legume species, several grasses), some that are used as medicinal or ornamental plants (e.g., genera *Monarda* L., *Mentha* L., and *Rhodiola* L.), many that are berries (e.g., raspberry, elderberry, blackberry, cranberry, gooseberry, blueberry, and currant), some that are crops (e.g., *Helianthus tuberosus*), many that are crop wild relatives (e.g., genera *Helianthus* and *Hordeum*), and many grass species that are interesting genetic resources from the perspective of food and agriculture. Weed species also deserve greater attention in this context. Several weeds have potential as genetic resources for utilization, including both native Canadian and naturalized species, and several are threatened by extinction. Some weeds have features similar to crops (convergent evolution with cultivated plants) which ensured their survival in farmers' fields prior to use of herbicides. An example for Canada is cow cockle (*Saponaria vaccaria* L.), which has recently received attention for cultivation in Western Canada due to its saponin content (Mazza et al. 1992; Willenborg and Johnson 2013) and earlier in the United States as a source of starch (Goering et al. 1966). The weed plants could easily become secondary cultivated plants, similar as oats and rye evolved from weeds in historic times (Vavilov 1926; Hammer et al. 1997). Also weeds with features showing divergent evolutionary features from cultivated plants can have the potential to become secondary cultivated plants, such as purslane (*Portulaca oleracea* L.) which is a popular salad plant among East Asian immigrant populations in Canada. They also represent a genetic resource for food and agriculture.

PGRC currently does not have a strong program focusing on ex situ conservation of native Canadian germplasm. The need to strengthen such activities was expressed

Table 2.2 Special collections of native Canadian germplasm at Plant Gene Resources of Canada (PGRC)

Name of collection at PGRC	Collector	Year of collecting	Type of material	Number of accessions
Main Collection of Native Canadian Plant Material	Plant Gene Resources of Canada and collaborators (Richard G. St-Pierre, Kenneth W. Richards)	Started 1996, ongoing	Many taxa, mostly from Western Canada, focus on forages, grasses, and species of economic potential	1964
ON & QC Rare Species Collection	Montréal Botanical Garden, Québec (Alain Meilleur, Frédéric Coursol, André Sabourin, Nadia Cavallin)	2006–2007	Black box collection, various species	90
Manitoba Orchid Society Collection	Manitoba Orchid Society (Richard G. St-Pierre)	2006–2010	Orchid seeds	58
Canadian Arctic Collection	Alaska Plant Materials Center, Palmer, Alaska, United States, (Stoney Wright)	2004, 2005	Emphasis on grasses with adaptation to coastal, subarctic conditions of Eastern Canada	870
Northwest Territories Collection	Aurora Institute, Northwest Territories, Canada (Pippa Seccombe-Hett, Annika Trimble, Ashley Mercer)	2005–2007	Wide range of native species native to the Northwest Territories	323
Symbios Research and Restoration Collection	Symbios Research and Restoration, Smithers, British Columbia (Philip J. Burton)	1996–1999	British Columbian species which have potential for habitat restoration	357
Native *Bromus* Collection	Agriculture and Agri-Food Canada (Bruce Coulman, Jacques Cayouette)	1993–1998	Mostly *Bromus* species from Western Canada	42

previously and is especially pressing since the vulnerability of native species to climate change is particularly high in Canada (Richards 2006). Systematic efforts to collect native Canadian plant diversity for ex situ conservation have only been conducted on a limited scale by collaborators of PGRC within Canada or from abroad. For example, in 2004 and 2005, Stoney Wright, a scientist from Alaska, United States, collected grasses with adaptation to arctic/subarctic conditions of Northern Québec, Nunavut, and the Northwest Territories. The Aurora Research Institute in the Northwest Territories collected seed of native species for use in reclamation projects (Aurora Institute 2016). Seed samples from such collection activities have been deposited in PGRC (Table 2.2), and selected grass and forage species from these collections have been regenerated and integrated into the active PGRC genebank collection. PGRC also preserves seeds of native Canadian material as security-backup samples (black box collections) for Canadian botanical gardens. These seeds are not tested for viability or researched in any other way by PGRC. Requests for material from all these special collections are treated differently than requests for germplasm from the main PGRC collections, and these special collections are not listed on the PGRC Internet website.

Interest by PGRC clients in native Canadian germplasm has been the reason for targeted collecting missions. The beer brewing industry in the Czech Republic was looking for native material of hops (*Humulus lupulus* L.) that was collected in Saskatchewan and Manitoba in 2003 for hop breeding and proved to be chemically distinct from European and other North American plants; the Canadian material was also found to have powdery mildew resistance (Patzak et al. 2010). The recent interest in false flax (*Camelina sativa* (L.) Crantz) has also resulted in collecting of crop wild relatives of this genus in Canada (Séguin-Swartz et al. 2013; Martin et al. 2015). However, limited capacities in the national Canadian genebank do not allow for ex situ conservation of all diversity of all native species that are plant genetic resources for food and agriculture, and priorities need to be established. In evolutionary terms, it is definitely preferable to maintain crop wild relative germplasm in situ in their native habitats, although access is facilitated by ex situ conservation. The ongoing discussion on the advantages, the disadvantages, and the feasibility of these two conservation principals is complex, has political implications, and started on the international level in the 1960s (Pistorius 1997; FAO 2016c).

Ornamental use of native species is also important. In rose breeding, two major rose cultivar series have been bred in Canada: The so-called series of Explorer roses bred by F.D. Svejda (1920–2016) are the basis for the series of Parkland roses and the Artist roses, which are based on introgression of adaptation traits from wild native species (Richer et al. 2007). Many other native trees, shrubs, and perennial and annual species have been identified that have potential value as ornamentals (Davidson 1995). Local industries promoting native plants for ornamental use can be found across Canada, and numerous websites exist for native plant societies in several Canadian provinces (North American Native Plant Society 2016). Currently, the main use for native species is in reclamation projects undertaken by the mining industry or other projects with massive landscape intervention.

2.3.2 Other Germplasm Collections of Native Canadian Crop Wild Relatives Within Canada

Working collections of fruit germplasm native to Canada exist at universities and at Agriculture and Agri-Food Canada research centers for research purposes. However, many fruit breeding programs have ceased. Some of these collections, such as strawberry, saskatoon berry (*Amelanchier* spp.), and rosehips (*Rosa* spp.), have been transferred to the Canadian national genebank system and are mostly preserved at the Canadian Clonal Genebank at Harrow, Ontario (PGRC 2016). The Canadian Food Inspection Agency maintains reference collections of fruit germplasm with emphasis on cultivars at Saanich, British Columbia; here, phytosanitary concerns are the primary purpose of the collection since virus diseases are regulated when such germplasm is transferred. However, the content of these collections is only accessible after inquiring directly to the respective institutions. A Canadian university collection containing native germplasm is accessible to the public from the Prairie Fruit Genebank established in the early 2000s at the University of Saskatchewan. This collection contains diverse material of native Canadian species such as blue honeysuckle (*Lonicera caerulea* L.), currant (*Ribes* spp.), raspberry (*Rubus idaeus* L.), and highbush cranberry (*Viburnum trilobum* Marsh.) (University of Saskatchewan 2016). The successful introduction of blue honeysuckle as a commercial crop in Western Canada is a recent example of utilization of native Canadian genetic resources (Bors 2009). Similarly, Fofana and Sanderson (2015) developed a new semidomesticated rosehip (*Rosa* hybrid) using native Canadian germplasm, and such germplasm was deposited at the Canadian Clonal Genebank of AAFC at Harrow, Ontario. In grapevine breeding, the Canadian native species *Vitis riparia* Mixch. has had great impact for introgressing adaptive traits into imported and less adapted grapevine (Reynolds et al. 2015).

A similar situation as for fruit germplasm exists for native Canadian grasses, forage legumes, and rangeland species. Native Canadian prairie vegetation has experienced a great decrease in area, from 61.5 M ha prior to European settlement to 11.4 M ha in recent years based on Statistics Canada Census 2006 and earlier published data (Bailey et al. 2010). This is partly due to policies that encouraged settlement and land utilization for annual crops (Bailey et al. 2010). Jefferson et al. (2005) identified some of the potential benefits of native plant species that have not received adequate attention. Cayouette et al. (1997) and Neufeld (2010) identified increasing interest in native plant species for reclamation, horticultural, and forage purposes. Some breeding of native species, which are registered as so-called Ecovars™, has existed in Western Canada since the 1990s (May et al. 1997). Initially, several AAFC research facilities and the University of Manitoba were involved with Ducks Unlimited Canada Ecovars™ in developing such material and a list of 24 suggested species of native grasses, legumes, and shrubs. As of 2016, AAFC Swift Current Research and Development Centre is the only facility still researching the collected material. Species that were registered from Swift Current include awned wheatgrass (*Elymus trachycaulus* (Link) Gould ex Shinners subsp. *subsecundus* (Link) Á. Löve & D. Löve), little bluestem (*Schizachyrium scoparium* [Michx.] Nash var. *scoparium*), northern wheatgrass (*E. lanceolatus* (Scribn. & J. G. Sm.) Gould subsp. *lanceolatus* var. *lanceolatus*), western wheatgrass (*Pascopyrum smithii* [Rydb.] Barkworth & D. R. Dewey), plains rough fescue (*Festuca hallii* [Vasey] Piper), and Canadian milkvetch (*Astragalus canadensis* L. var. *canadensis*). Initially, this effort focused on revegetation needs, but continued research has focused on agricultural needs. This ongoing research has found characteristics within the native plant species that provide benefits beyond forage use. Purple prairie clover (*Dalea purpurea*), for example, was found to have a condensed tannin profile that decreases the shedding of *Escherichia coli* bacteria in cattle (Jin et al. 2015). Winterfat (*Krascheninnikovia lanata* [Pursh] Meeuse & Smit), a winter forage shrub, is noted as improving digestibility of low-quality plant material when ingested together (Schellenberg 2005). Utilization of the native legume *Thermopsis rhombifolia* (Nutt. ex Pursh) Richardson is also being investigated for medical purposes such as anticancer effects in humans (Kerneis et al. 2015). The relevance of native perennial bromegrass as forage has been emphasized (Cayouette et al. 1997). As stated previously, the potential of native Canadian plant species is largely unexplored, but the preceding examples provide a small insight into the potential benefits of further exploration, hopefully before the resource no longer exists.

Botanical gardens have an important role in conservation biology. Canada has about 100 botanical gardens, arboreta, and related facilities, and many of them are active in the Botanic Gardens Conservation International initiative (BGCI 2016). For crop wild relatives and for accessing germplasm, the following institutions are most relevant: Devonian Botanical Garden, Edmonton, Alberta; Montreal Botanical Garden, Montreal, Québec; Royal Botanical Gardens, Burlington-Hamilton, Ontario; and UBC Botanical Garden, Vancouver, British Columbia. The Montreal Botanical Garden has projects that relate in particular to ethnobotany involving indigenous people from Canada. The Canadian Botanical Conservation Network (CBCN) is a project of the Royal Botanical Gardens, which was established in 1995 and has the objective to coordinate conservation activities among different institutions. It is linked to the North American botanic garden strategy for plant conservation (BGCI 2016) and provides information into the COSEWIC species at risk assessments for Canada. Botanical gardens in Canada do not systematically research the aspects of utilization of native plants for food and agriculture. In addition, intraspecific diversity, which is the genetic diversity of great relevance for utilization, is rarely featured by botanical gardens because mostly the species as such is the category of concern and not the diversity within a species. However, this aspect of genetic diversity has recently gained some momentum both in botanical gardens within Canada and internationally.

2.4 In Situ Conservation

In situ conservation is of great relevance for native Canadian species. In 2015, about 10.6% of Canada's terrestrial area was protected and under governance of the federal or provincial governments (Fig. 2.6, Environment and Climate Change Canada 2016b). However, the locations of these protected areas are either far from human settlement or of high recreational value, both of which impact biodiversity. The areas of greatest relevance for agriculture, such as the arable prairie areas, the mixed wood plains of Southern Ontario, and the regions along the St. Lawrence River, cover less than 2% of the area protected (Anonymous 2014). Therefore, crop wild relatives located in these regions benefit little from this type of protection. A comparison of the species rich (dark red) areas in Fig. 2.4 in Southern Ontario with the lack of protected areas in the same regions in Fig. 2.6 illustrates this discrepancy. Some areas are floristically of great relevance, such as the alvars in Western Québec

Fig. 2.6 Distribution and size of terrestrial (land and freshwater) protected areas in Canada and marine protected areas in 2015. (Environment and Climate Change Canada 2016c)

and in Ontario, which are open habitats on thin soil over calcareous beds. They harbor a lot of rare native species that are genetic resources.

Climate change impacts all biodiversity and agriculture massively. Urbanization in the densely settled areas is another challenge. Agroecosystems for in situ conservation of crop wild relatives are important to consider and a fairly new concept. In addition to the protected areas, there are 18 UNESCO biosphere reserves in Canada. These are not static in their conservation work but integrate sustainable human activities and pay particular respect to indigenous people (Canadian Biospheres Reserves Association 2017). Populations of crop wild relatives are affected by all measures taken by agriculture. Great selection pressure comes from pesticide use. Many weeds that are crop wild relatives became rare species today. Weeds are rarely seen as being worthy of conservation because of two tendencies: botanical science neglects them and agricultural science combats them. Carr (1993) pointed out the potential of stinkweed (pennycress, fanweed, *Thlaspi arvense* L.), a very common weed of Western Canada. The biofuel industry has started to work with this species (Dorn et al. 2015). PGRC has 23 accessions of this species in its collection and contributed to research by providing such germplasm.

The possibility of gene flow integrating herbicide tolerance into weeds and crop wild relatives is a reality in Canada, and this phenomenon is accelerating. An example was provided by Séguin-Swartz et al. (2013) for species within the genus

Camelina that are naturalized in Canada. Martin et al. (2015) showed that the more distant *Capsella bursa-pastoris* (L.) Medik., which is also a neophyte in Canada, produces offspring when pollinated with the cultivated species *Camelina sativa* (L.) Crantz. Due to common agricultural practice, the selection pressure for developing herbicide tolerance in Canada is enormous and affects the diversity of weeds and crop wild relatives. Such resistance was found in many weeds in Western Canada including crop wild relative genera of grasses (*Bromus* L.*, Hordeum* L.*, Setaria* P. Beauv.*, Elymus* L.*, Avena*) and dicotyledonous plants (*Lepidium* L.*, Amaranthus* L.*, Sinapis* L.) (Beckie et al. 2013). Some of these weed populations may be used as sources for such traits in breeding of the related cultivated species. The most prominent crop wild relative in Canada is *Avena fatua*. It is not a native species but is decidedly naturalized, widely spread, and well preserved in in situ/on-farm situations. PGRC maintains 644 accessions of this species. It is part of the primary genepool for *A. sativa* L., and in Canada the two species form a crop-weed complex influencing each other by introgression (Van Raamsdonk and Van der Maesen 1996). The evolutionary response to modern agriculture has resulted in herbicide tolerances in *A. fatua*, but the wild oat has also been used as a crop on its own (Beckie et al. 2012).

Botanical inventories of species differentiating according to rareness of species in Canada exist. An excellent source for species occurrences in Canada was provided by Brouillet et al. (2010). Citizen scientists and naturalists contribute to knowledge about the Canadian flora. An example of a useful guide to wildflowers for the province of Saskatchewan is provided by Lee (2017). A recent review emphasized the lack of representations of crop wild relatives in genebank collections on the global scale and pointed out the gaps in Canada (Castañeda-Álvarez et al. 2016). Their study did not assess the conservation status of the species (whether they are threatened), which would be important information to justify the major effort of ex situ conservation. Some habitats such as the native prairie are becoming rare, and their species need attention. Examples of crop wild relatives are the sunflower species (Fig. 2.7), native plants with medicinal uses (Fig. 2.8), and native grasses (Fig. 2.9).

Having the utilitarian aspect of genetic resources in mind, it would be helpful to differentiate among wild non-utilized species, wild utilized species, crop wild relatives, weeds, naturalized species, and cultivated species or taxa. The genepool concept of Harlan and De Wet (1971) could be used which groups wild species according to their capability to intercross with a cultivated taxon into a primary, secondary, and tertiary genepool. Not all species in the same genus as a crop are necessarily relevant as genetic resources. For example, all native Canadian *Hordeum* species are genetically very distant from the cultivated *H. vulgare* L. and cannot be used in traditional plant breeding; if anything, they belong to the tertiary genepool for barley breeding. Taxonomic studies are needed in many species to clarify these relationships. Another challenge is that many native species, in particular rangeland species, are not known for their potential for food and agriculture.

It is noteworthy that native grassland species and populations have become rarer and disjunct during the course of agricultural settlement in Western Canada. Management of native grassland populations has also changed considerably.

Fig. 2.7 Aspect of *Helianthus nuttallii* Torrey and A. Gray, a crop wild relative of sunflower, at Eagle Creek, Saskatchewan. (Photo: Maureen Carter)

Fig. 2.8 Aspect of wild bergamot (*Monarda fistulosa* L.), a medicinal and ornamental plant, at Eagle Creek, an *Urstromtal* (meltwater valley) in Saskatchewan. (Photo: Maureen Carter)

Fig. 2.9 June grass (*Koeleria macrantha* [Ledebour] Schultes), a native grass, at Grasslands National Park, Saskatchewan. (Photo: Branimir Gjetvaj)

Naturally occurring fires were more frequent prior to settlement, and fire protection measures have changed the habitats. In addition, the Canadian government has reduced its engagement in active management of native rangelands in Western Canada during the last decade. Government-owned land (provincial and/or federal) and pastures administered by the Prairie Farm Rehabilitation Administration (established in 1935 as a result of the prolonged drought of the 1930s to deal with soil erosion and water development for agricultural purposes (Gilson 2013)) are estimated to have once covered 1.9 M ha, of which 1.0 M ha was viewed as native grassland (Bailey et al. 2010). With the divestiture of the land to non-federal entities to be completed by 2017, the conservation role of these lands is in question. The Province of Saskatchewan was the largest recipient of the divested lands and announced the phasing out of the Saskatchewan Pastures Program starting in 2017 (Robinson 2017), thus adding additional angst in regard to how these remnants of native rangelands will be managed. The natural grasslands are known for being hardy and drought resistant and are considered key forage for livestock production (Bailey et al. 2010). The species within these natural grasslands could hold the key for adaptation to predicted climate scenarios.

2.5 General Assessments Regarding the Conservation and Utilization of Native Canadian Plant Diversity

Many authors have identified the relevance of combining conservation and utilization of biological diversity (Heywood 1999b). Others have emphasized the fact that we have only started to explore the potential of wild species for utilization (Balandrin et al. 1985; Small 1995, 1999). The importance not only of living germplasm collections but also of reference collections such as herbaria has been emphasized (Council of Canadian Academics 2010). The vascular plant herbarium maintained by Agriculture and Agri-Food Canada in Ottawa (DAO Herbarium) is a unique source of information and has been instrumental in providing information about the diversity of native Canadian crop wild relatives and for collecting native crop wild relatives in Canada (Agriculture and Agri-Food Canada 2016). The 1.6 million specimens in this herbarium have been assembled since 1886 with an emphasis on weeds, crops, and crop wild relatives (Anstey 1986). The relevance of this collection for identification, localization, and eventual utilization is enormous and its potential by far not realized (Mitrow and Catling 2012; Small 2011). There are only very few herbarium collections in the world having such an emphasis on crop plants, crop wild relatives, and weeds. Digitization of the specimens of this and other herbarium collections in Canada is ongoing and has greatly improved access to these resources. Combining conservation and utilization remains a challenge. Crop wild relative species are obtaining more recognition in fundamental botanical science than in applied agricultural science, but the utilization aspect is often overlooked.

Molecular assessments of diversity in native species enhanced the understanding of diversity and can assist in conservation activities. A molecular research program on native Canadian grass species in association with PGRC has existed since 2002 in collaboration with other scientists in AAFC, National Parks, Environment Canada, the University of Saskatchewan, and Ducks Unlimited to conduct 11 research projects on genetic diversity of more than 14 native grass species (Fu et al. 2005a; Fu and Thompson 2006; Liu et al. 2013; Biligetu et al. 2013). Observations of diversity changes over time and space are helpful to make conservation decisions for such plant material (Ferdinandez et al. 2005; Fu et al. 2005b; Qiu et al. 2009). This advanced knowledge about native grass species contributed to Canadian forage breeding and native grass conservation and restoration. Due to these activities, PGRC acquired more than 100 accessions of these native grass species for PGRC's native grass collection. Cytological studies showed a great divergence between diploid and tetraploid races of small cranberry (*Vaccinium oxycoccos* L. s.l.) native to Canada (Smith et al. 2015). Chemical evaluations of native *Desmodium* species at PGRC to assess saponins in the foliage have been conducted and guide users to useful germplasm (Taylor et al. 2009). These accomplishments and the efforts of PGRC and cooperating institutions in collecting germplasm of native Canadian species as shown in Table 2.2 are important to build on for future conservation and utilization activities for such germplasm in Canada. The major contributions by the AAFC staff associated with the DAO Herbarium in Ottawa are also a critical building block to ground future work on.

A unique plant native to Canada and the United Sates is wildrice (*Zizania palustris*, *Z. aquatica*). In Canada, it is collected from the wild for utilization, while in the United Sates, some breeding has occurred to reduce seed shattering (Small 2014). The seeds are recalcitrant (Aiken et al. 1988), which means they cannot be dried and easily stored in a genebank. In situ conservation is the only feasible strategy for this germplasm, but the natural habitats are in decline. We lack knowledge of the genetic diversity in the species of *Zizania* native to Canada. A database on Brassicaceae of Canada is available on the Internet site of the Canadian Biodiversity Information Facility (CBIF) and was created because of the relevance of Brassicaceae oilseeds and mustards for the Canadian economy (Warwick et al. 2016). Native berries and small fruits are very important examples of native Canadian species contributing to a major crop (Hancock and Luby 1993; Catling and Cayouette 1994). Some berries are quite rare and only known in the very northern hemisphere, such as *Rubus chamaemorus* L. (Beaulieu et al. 2013).

Native Canadian *Helianthus* species are also important (Kantar et al. 2015); of these, *H. tuberosus,* naturalized in Canada, is well represented at the Canadian genebank (Diederichsen 2010). Many of the Canadian species that are genetic resources also occur in the United Sates, and a coordinated approach between the two countries is beneficial. However, there are many knowledge gaps, and native Canadian species alongside with alien species occurring in Canada should be further studied in their occurrence and potential use. An example is the genus *Lactuca* L. (Lebeda et al. 2009).

Weeds are well-studied as problems for agriculture in Canada, and their distributions are monitored (CFIA 2008). Darbyshire (2003) provided a comprehensive list of 872 weed species for Canadian agriculture. Many of these weed species were introduced, and some are cultivated plants in other parts of the world, such as *Fagopyrum esculentum* Moench and *F. tataricum* (L. Gaertn.). The great importance of weeds as genetic resources is frequently overlooked (Hammer et al. 1997). Again, exceptions exist, such as the recognition of ruderal and weedy hemp as genetic resources (Small et al. 2003) or the recent activities in cow cockle (Willenborg and Johnson 2013). Possibly, weeds are particularly in need of ex situ conservation. They are adapted to cultivated habitats and pressed hard to survive in modern agriculture, where those surviving may eventually all develop herbicide tolerances.

There is no coherent picture when it comes to the study, conservation, and utilization of native and naturalized Canadian plant species for food and agriculture. The size of the country, resulting in great distances among scientific and research institutions, and the fact that the agricultural areas are very concentrated in a few regions are contributing factors to this. But also in strictly botanical issues, incoherence can be observed. For example, for all of Canada, 196 plant species are listed with a conservation status "of concern" (COSEWIC 2017). However, an assessment for the province of Québec reports a total of 409 vascular plant species as being of concern for this province alone (Tardif et al. 2016). The potential residing in native Canadian plants is enormous, and there are good reasons to explore in particular Canada's native fruit, forages, and rangeland species. Reclamation projects and forestry are the most advanced in the use of native Canadian plant species.

2.6 Conclusions, Challenges, and Opportunities to Conserve Native Canadian Plant Genetic Resources

Native Canadian plants include species used for food, forages, medicinals, ornamentals, forestry, or spiritual reasons. Many are used or have potential for multiple purposes, and there is overlap with species that are crop wild relatives. Many of these species are relevant genetic resources for food and agriculture. Some native or naturalized weed species have potential for utilization, and some are threatened by extinction and are subject to genetic changes due to the industrialization of agriculture. Very few Canadian plant species are likely to become significant agriculturally. Identifying the species with the most potential, researching them, and ensuring the conservation of their genetic diversity are the first step necessary from a genetic resources viewpoint. Canada needs a better understanding not only of the native plant genetic resources that are relevant to the Canadian agricultural sector but also of those that are important from a global perspective. International cooperation in this area will be beneficial since it will spread the burden of expensive ex situ conservation and help share experience in the in situ and on-farm conservation. For example, many of the berry crops in Canada are also relevant in Scandinavia, Russia, and the United States.

We still lack knowledge of phenotypic and genetic properties relevant to utilization of many native Canadian species, although Davidson (1995) provided a baseline for Canada. Due to urbanization and environmental change, we may lose diversity in natural habitats that hold options for the Canadian agricultural sector. In the dominating industrialized agricultural ecosystems, selection pressure is influencing the species and genetic diversity at an accelerating rate. Collections of living germplasm of native species have been assembled at Plant Gene Resources of Canada (PGRC), and research activities have been initiated. Some of these collections are in poor condition and need regeneration.

PGRC should play a role in making decisions when in situ monitoring of native diversity is sufficient and when expensive ex situ conservation at PGRC is not warranted or simply not feasible. Genebanks do not need to preserve all diversity of crop wild relatives and indeed lack the capacity to do so. Priorities for ex situ conservations need to be set based on a strategic and pragmatic approach. The complementarity of in situ and ex situ conservation needs to be established. In many cases, monitoring of native populations of crop wild relatives is preferable to ex situ conservation. The latter should only be the last resort to protect a wild species or a certain ecotype of a wild species from extinction. Despite the facilitated access for users when properly preserved in genebanks, the evolutionary implications of ex situ vs. in situ conservation are particularly important in wild species, and protection of natural habitats is more effective for wild species than ex situ conservation. For some obligatory weeds that cannot survive as wild plants, ex situ conservation may be appropriate. It is important to build on the efforts made by PGRC in the past when the program on native Canadian diversity was more active, which was a result of the first response by the Canadian government and AAFC to the CBD in the 1990s.

Hammer (2003) suggested the concept of the "integrated genebank" which would take part in all areas on conservation and utilization of genetic resources. Possibly, genebanks need to be more active, and in fact a concept of an "integrating genebank" needs to be implemented. Such a proactive genebank in the spirit of the International Treaty on Plant Genetic Resources for Food and Agriculture was also outlined by Louwaars (2012). It is desirable to establish more case studies demonstrating diversity of native Canadian species that are crop wild relatives, have potential, or are wild utilized species. The assumption is that many options are not yet explored. Future collecting of herbarium specimens and germplasm would benefit from coordination. It seems appropriate that Agriculture and Agri-Food Canada takes the lead in this area. Such initiative relates to commitments Canada made in international agreements including the CBD and presently under discussion by the Commission on Genetic Resources for Food and Agriculture of the United Nations (FAO 2016c) which suggested developing national strategies for conservation and utilization of crop wild relatives. PGRC should intensify interactions with indigenous Canadian people to support the conservation and utilization of the native Canadian diversity they have utilized for many centuries. Also, it will be important to enhance awareness for biodiversity-related issues not in an abstracts or theoretical way, but by showing and communicating how every citizen can impact the diversity that will be available for the future.

2.7 Conclusions

- For economic reasons and from a conservation perspective, there is an urgent need to develop a revised and rigorous Canadian national strategy for conservation and utilization of native Canadian plant diversity that is of relevance to the Canadian agricultural sector. Such a strategy should be developed in coordination among several national stakeholders and with other countries.
- The actual and potential benefits of native Canadian plant diversity to agriculture and for other uses need to be better understood and documented.

- Enhanced cooperation of Agriculture and Agri-Food Canada with Environment and Climate Change Canada, provincial partners, botanical gardens, First Nations groups, plant breeders, universities, and non-governmental organizations would be beneficial.
- Investing in a strategic and integrative approach and building on the complementarity between in situ and ex situ conservation will secure opportunities for agriculture and benefit all stakeholders.

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Chapter 3
Crop Wild Relatives in Mexico: An Overview of Richness, Importance, and Conservation Status

J. Fernando De La Torre S., Rosalinda González S., E. Judith Cruz G., J. Manuel Pichardo G., Martín Quintana C., Aremi R. Contreras T., and Jorge Cadena I.

Abstract Mexico is recognized as an important center of plant diversity, with a high proportion of this diversity being endemic to the country. It is also considered a center of origin, domestication, and diversification of many crops of national, regional, and global importance, and there is also a high diversity of crop wild relatives (CWR). CWR are important sources of genetic variation and can be used for the genetic improvement of crops. In this introductory chapter, an insight about the importance of Mexican CWR diversity and their conservation status is given. We cover the general outline of the national legislation on the conservation, utilization, and sharing of plant genetic resources, with a description of the main stakeholders responsible for the definition and implementation of conservation actions. An assessment of the ex situ and in situ CWR conservation strategies is also carried out, including germplasm banks, arboreta, botanic gardens, protected areas, and genetic reserves. We summarize the conservation status of CWR of some of the most important crops in Mexico and Mesoamerica, i.e., maize, beans, pepper, cotton, potato, tomato, and avocado. We also review the effects of climate change and other threats on the diversity of CWR in the region. We finalize with an analysis of challenges and opportunities for the conservation of CWR. The generation and dissemination

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J. F. De La Torre S. (✉) · E. J. Cruz G. · J. M. Pichardo G. · M. Quintana C.
A. R. Contreras T.
Centro Nacional de Recursos Genéticos, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, México (National Genetic Resources Centre, National Institute of Forestry, Agriculture and Livestock Research), Tepatitlán de Morelos, Jalisco, Mexico
e-mail: delatorre.fernando@inifap.gob.mx

R. González S.
Servicio Nacional de Inspección y Certificación de Semillas, Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (National Seed Inspection and Certification Service, Secretariat of Agriculture, Livestock, Rural Development, Fisheries and Food), Coyoacán, Mexico City, Mexico

J. Cadena I.
Colegio de Postgaduados (Postgraduate College), San Luis Potosí, Mexico

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of knowledge on the importance of the CWR diversity in the country is vital to achieve the long-term conservation and sustainable utilization of these valuable genetic resources.

Keywords Crop wild relatives · Plant genetic resources · Conservation · In situ conservation · Ex situ conservation · Mexico · Germplasm

3.1 Introduction

Mexico is a mega-diverse country (Sarukhán et al. 2009), representing 1.5% of the world's land mass yet harboring 10% of the known biodiversity. Among countries, it ranks fourth for having the most species diversity (Mittermeier and Mittermeier 1992) and is among the richest countries for endemism (CONABIO and SEMARNAT 2009). This genetic richness is the result of its latitudinal position and geographic landscape that bring special climatic and physiographic characteristics which determine the complexity of Mexico's ecosystem and biological diversity (Sarukhán et al. 1996). As a mega-diverse country, Mexico has made an important commitment to safeguard its biodiversity for present and future generations (Benitez-Díaz and Bellot-Rojas 2003). The Mesoamerican region, which includes Mexico, as well as Belize, Guatemala, Honduras, El Salvador, Nicaragua, Panama, and Costa Rica, is one of the centers of origin and domestication of many crops (many of them of global use) and their crop wild relatives (CWR) (Harlan 1971). Although this chapter focuses mainly on Mexico, we present data on the Mesoamerican region (from central Mexico to Northern Costa Rica) since much research has been done on the region.

Mexican plant diversity accounts for more than 25,000 species of vascular plants (Llorente-Bousquets and Ocegueda 2008), and it is estimated that 5000–7000 species, domesticated and wild, are utilized by humans in the Mesoamerican region (Casas et al. 2007). In Mexico, it is reported that 600–700 plant species are utilized by indigenous groups practicing in situ management systems like systematic gathering and protection (Caballero et al. 1998) and at least 142 species have been domesticated and the majority of them have CWR (Perales and Aguirre 2008). Vincent et al. (2013) reported the possible existence of 109 priority CWR in Mexico, resulting from the analysis of 92 global priority crops. Figure 3.1 illustrates CWR richness in Mexico based upon 304 high-priority CWR species identified in this book. It is uncertain if this number could be larger since a comprehensive field study has not been done in Mexico to systematically inventory all the CWR of crops whose center of origin, domestication, or diversification is Mexico.

There are, however, a number of studies describing the CWR of important crop genera of Mexico, such as *Zea* L. (maize), *Phaseolus* L. (bean), *Cucurbita* L. (squash), *Gossypium* L. (cotton), *Capsicum* L. (pepper), *Solanum* L. (tomato, potato), *Sechium* P. Browne (chayote), *Amaranthus* L. (amaranth), *Persea* Mill. (avocado), *Vanilla* Mill. (vanilla), *Opuntia* Mill. (prickly pear cactus, nopal),

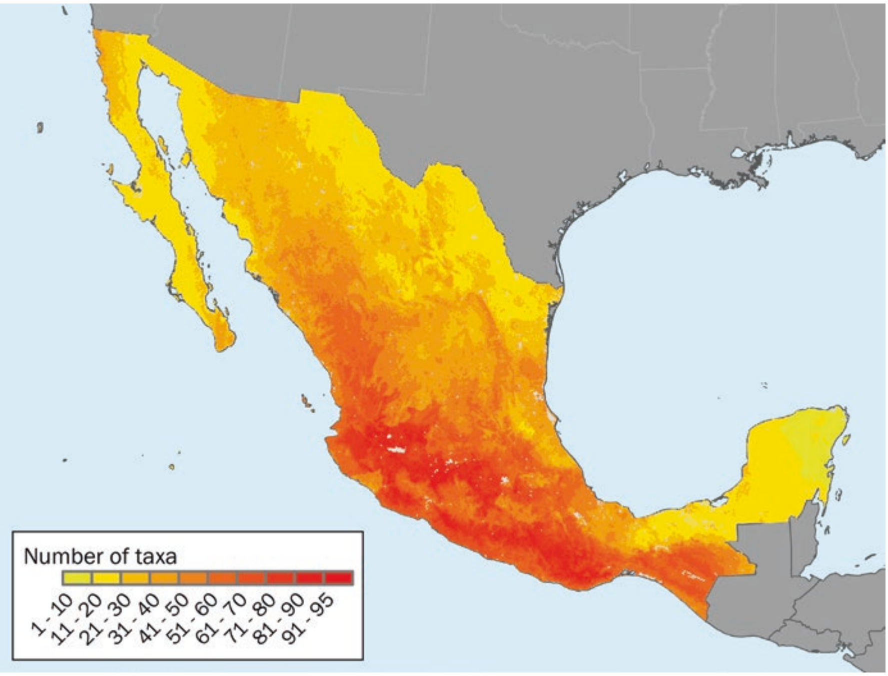

Fig. 3.1 Estimated potential richness of taxa in Mexico that are crop wild relatives based on 304 species using data of climatic and edaphic similarities of the assessed area with herbarium and genebank reference localities. Richness of potential distributions of crop wild relative taxa decreases from red to yellow. The method for generating the map and a list of reference data providers is given in Appendix 1

Physalis L. (ground cherry), *Tagetes* L. (marigold), *Ananas* Mill. (pineapple), *Theobroma* L. (cacao), and *Ipomoea* L. (sweet potato), among others. For example, the genus *Phaseolus* has 63 species; 52 of them are present in Mexico, but only 5 are domesticated and 5 more are used by people as wild species (information provided by A.O. Delgado S., in: Perales and Aguirre 2008). An important genus, *Zea*, has 1 domesticated species (*Zea mays* L. subsp. *mays*) and 9 CWR (teosintes), 6 originating in Mexico, 2 in Guatemala, and 1 in Nicaragua, and 16 species of the genus *Tripsacum* L. (a genus closely related to *Zea* and whose species are able to cross with *Zea* species to produce fertile plants), 14 of which are present in Mexico. It is important to mention that the main relevance of *Tripsacum* species is their potential as forages in the Mesoamerican region (J. J. Sánchez G., in: Perales and Aguirre 2008; Villanueva-Avalos et al. 2015). A project aiming to identify and safeguard important CWR at the national and regional levels across the Mesoamerican region is being currently conducted by institutions and organizations from El Salvador, Guatemala, Honduras, Mexico, and the United Kingdom and will produce more solid information on the inventory of CWR at the national level (www.psmesoamerica.org).

3.2 General Overview of National Plant Genetic Resources Conservation and Use Policies in Mexico

Mexico is part of the Convention of the International Union for the Protection of New Varieties of Plants (UPOV), which was established in 1961. To date, 74 countries are members (UPOV 2017). In Mexico the Convention was implemented through the Federal Plant Varieties Law, based on the 1978 Act of UPOV (González-Santos et al. 2015). The World Trade Organization's Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS) regulates the intellectual property of plant genetic resources because it identifies patentability constraints that exclude plants and animals other than microorganisms, and biological processes for their production, but allows the members of the organization to provide protection to all plant varieties through patents or a sui generis system or a combination of these (Martínez-Prat 2003).

Mexico is also a party to the Convention on Biological Diversity (CBD) and its protocols including the Cartagena Protocol on Biosafety (CPB), the Nagoya-Kuala Lumpur Protocol on Liability and Redress (NKLP) to the CPB, and the Nagoya Protocol on Access and Benefit Sharing (NPABS) (CDB 2017). The implementation of CPB in Mexico occurred with the Biosecurity of Genetically Modified Organisms Law (DOF 2005). Concerning NPABS, the Federal Official Gazette published in October 2014, the "Decree promulgating the Nagoya Protocol on access to genetic resources and fair and equitable sharing of benefits arising from their Utilization to the Convention on Biological Diversity, adopted in Nagoya on 29 October 2010" (DOF 2014). With the implementation of the CBD, a national biodiversity strategy was defined in 2000. In 2016, the National Biodiversity Strategy and Action Plan (NBSAP) (2016–2030) was implemented. The NBSAP comprises six strategic approaches: (1) knowledge; (2) conservation and restoration; (3) sustainable use and management; (4) attention to pressure factors; (5) education, communication, and environmental culture; and (6) mainstreaming and governance (CONABIO 2016). The most closely related to plant genetic resources for food and agriculture (PGRFA) is the Mexican Plant Conservation Strategy 2020–2030, which applies to all cultivated and wild plant species and focuses on six goals including (i) conservation in situ and ex situ; (ii) sustainable use; (iii) generation and diffusion of knowledge; (iv) environmental education and culture; (v) prevention and control of threats; and (vi) restoration of degraded ecosystems (CONABIO 2012). In addition, it is related to the National Strategy on Invasive Species in Mexico, which proposes actions to prevent, detect, and reduce the risk of introduction, establishment, and spread of invasive species that impact productive activities and displace native species (Comité Asesor Nacional sobre Especies Invasoras 2010). PGRFA also relates to the United Nations Framework Convention on Climate Change (UNFCCC, www.unfccc.int) and the Convention to Combat Desertification (UNCCD, www.unccd.int). Some of the obligations of this convention are enforced in Mexico through the General Law of Climate Change (DOF 2012), which was last revised in 2016.

The International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) issued by the United Nations Food and Agriculture Organization (FAO) in 2002 is the specific international instrument for the subject of PGRFA (FAO

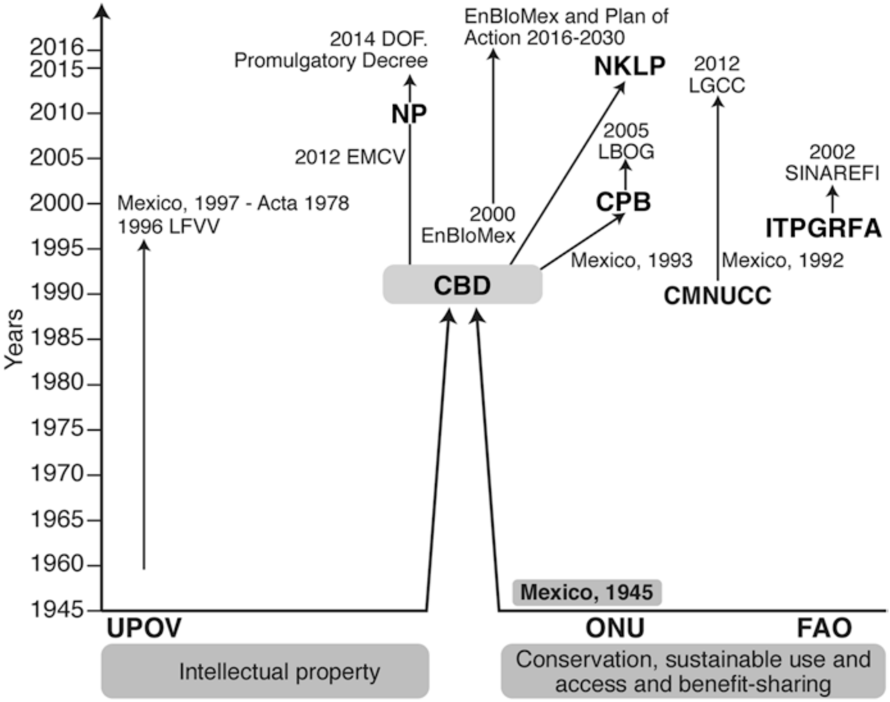

Fig. 3.2 International conventions and treaties related to plant genetic resources. It includes the main legal instruments or public policies implemented in Mexico. International Union for the Protection of New Varieties of Plants (UPOV), Federal Law on Plant Varieties (LFVV), World Trade Organization (WTO) Agreement of the World Trade-Related Aspects of Intellectual Property Rights (TRIPS), Convention on Biological Diversity (CBD), Official Gazette of the Federation (DOF), Mexican Plant Conservation Strategy (EMCV), National Biodiversity Strategy of Mexico and its Action Plan 2016–2030 (ENBioMex) Nagoya Protocol (NP), Cartagena Protocol on Biosafety (CPB), Nagoya-Kuala Lumpur Protocol on Liability and Compensation (NKLP), United Nations Framework Convention on Climate Change (UNFCCC), Law on Biosafety of Genetically Modified Organisms (LBOGM), Law on Climate Change (LGCC), National System of Plant Genetic Resources for Food and Agriculture (SINAREFI) (Based on Raustiala and Víctor 2003)

2009). Although Mexico is not a party to the Treaty, the Secretariat of Agriculture, Livestock, Rural Development, Fisheries, and Food (SAGARPA), through the National Seed Inspection and Certification Service (SNICS), implemented a government policy (Corzo 2013), the National Program of Plant Genetic Resources for Food and Agriculture, supported by the Law on Sustainable Rural Development, which is the only law in Mexico addressing the term “plant genetic resources.” In its Article 102, Section I, it is stipulated: “Establish and if necessary propose, in conjunction with other agencies and related institutions, policies, actions and agreements on conservation, access, use and integrated management of plant genetic resources” (DOF 2001), last revised in 2012. Figure 3.2 summarizes the main conventions and treaties related to plant genetic resources in regard to conservation, sustainable use, and intellectual property.

In addition, there are laws related to PGRFA including the Industrial Property Law (DOF 1991), last revised in 2016, for the registration of patents; the Federal Law of Production, Certification, and Trade of Seeds (DOF 2007), for the registration of varieties (DOF 1988) and last revised in 2017; the General Law of Wildlife (DOF 2000), last revised in the National Catalog of Varieties of Plants, for collection permits; and the General Law of Ecological Equilibrium and Protection to the Environment in 2016, for the conservation and use of wild relatives of domesticated species. In addition, legislation that cover general aspects are the Agrarian Law (DOF 1992), last revised in the DOF in 2017; the Federal Plant Protection Law (DOF 1994), last revised in the DOF 2011; the Organic Products Law (DOF 2006); the Law on the Promotion and Development of Bioenergetics (DOF 2008); and the Law of the National Commission for the Development of Indigenous Peoples (DOF 2003), last revised in 2016.

The abovementioned legislation is implemented mainly by the SAGARPA, coordinated by the Sub-Directorate of Genetic Resources (SAGARPA 2017); the National Seed Inspection and Certification Service (SNICS), coordinated by the Directorate of Plant Genetic Resources (SNICS 2017); the Ministry of the Environment and Natural Resources (SEMARNAT), coordinated by the Directorate for Regulation of Biosafety, Biodiversity and Genetic Resources (SEMARNAT 2017); and the National Commission for the Knowledge and Use of Biodiversity (CONABIO), through the Coordination of Biological and Genetic Resources (CONABIO 2017).

Another institute that contributes to the implementation of legislation is the National Commission for the Development of Indigenous Peoples (CDI 2017). Also in Mexico, there are public research institutions that have specific lines of work in plant genetic resources, such as the National Institute of Forestry, Agriculture and Livestock Research (INIFAP 2012), Chapingo Autonomous University (UACh 2017), Postgraduate College (CP 2017), Antonio Narro Agrarian Autonomous University, and the University of Guadalajara (UDG 2017), among other academic institutions.

Regarding current national government policies, the subject "Plant Genetic Resources" is not mentioned; however, in some objectives and goals, it is implicit, for example, the National Development Plan, in goal IV, objective 4.10, Strategy 4.10.1 (PND 2013); the Sectorial Program of the SAGARPA, objective 4, Strategies 4.3 and 4.4 (DOF 2013a); and several strategies of the Sectorial Plan of the SEMARNAT (DOF 2013b). Furthermore, CONABIO implements biological, ecological, or conservation corridors within existing protected areas (national parks, biological reserves) or the remnants of the original ecosystems and maintains their connectivity through productive activities in the intermediate landscape allowing the flow of species (CONABIO 2017). Finally, the Mexican Society of Plant Genetics (SOMEFI), founded in 1965, publishes scientific research on plant genetic resources in the journal *Revista Fitotecnia Mexicana* (SOMEFI 2017).

3.2.1 Implementation of the National System of Plant Genetic Resources for Food and Agriculture (SINAREFI)

As noted above, the only public policy of governance (Corzo 2013) specific to the subject of plant genetic resources was implemented by SNICS and it is referred to as the National System of Plant Genetic Resources for Food and Agriculture program (SINAREFI). Through this policy, a coordination mechanism for the conservation and sustainable use of plant genetic resources was established, warranting the fair and equitable distribution of the benefits derived from their use. The SINAREFI was implemented to address the main premises identified in the reports prepared by Ramirez et al. (2000) and Molina and Córdova (2006). These authors pointed out that Mexico had the capacity in terms of infrastructure and technical assistance but lacked coordination. This resulted in duplication of projects, lack of crop prioritization, and legal and technical gaps that did not protect germplasm against bio-piracy and exploitation of Mexico's genetic heritage (González-Santos 2016).

SNICS implemented at the national level the "Strategy for the Conservation and Sustainable Use of Plant Genetic Resources for Food and Agriculture" based on the First FAO Global Plan of Action for Plant Genetic Resources for Food and Agriculture (FAO 1996). This includes four strategic areas: (1) in situ and (2) ex situ conservation, (3) use and enhancement, and (4) capacity building. Crop networks were developed that were interdisciplinary and interinstitutional to ensure the involvement of all actors. The SNICS integrated a platform with more than 60 participating institutes with 400 researchers and more than 500 participating producer directors grouped into 46 networks by crop (González-Santos et al. 2015).

SNICS developed a National Plan of Action for the Conservation of Plant Genetic Resources for Food and Agriculture (PANREFI) based on the needs identified in reports produced in 2000 and 2006 and the first Global Plan of Action for Plant Genetic Resources for Food and Agriculture (FAO 1996), issued by the Food and Agriculture Organization of the United Nations (FAO). The PANREFI consists of 4 strategic areas and 20 lines of action (Table 3.1). For the implementation of PANREFI, SINAREFI integrated networks by crop, in order to take advantage of capacity and infrastructure and avoid duplication of work (González-Santos 2016).

An analysis of priorities was carried out using three criteria: importance in food and agriculture; centers of diversity, origin, or diversification in Mexico; and socioeconomic impact. From these criteria, 44 native crops were grouped into the following thematic networks: basic and industrial, fruits, ornamentals, emerging crops (underutilized plant species), and vegetables (Table 3.2) (González-Santos 2016).

Table 3.1 Strategic areas and lines of action of the PANREFI

In situ conservation	Ex situ conservation	Use/enhancement	Capacity building
Inventory	Collections maintenance	Characterization	Network coordination
Participatory breeding	Regeneration	Genetic improvement	Network promotion
Assistance in catastrophic events	Recollection	Diversification promotion	Information systems
Promotion of NUS	Enhancement of conservation activities	Development of NUS	Monitoring and alert systems
		Seed production	Teaching and training
		Creation of new markets	Public awareness

Table 3.2 Priority crops of the PANREFI grouped into thematic networks (González-Santos 2016)

Agave (*Agave* L.)[B]	Dragon fruit and pitaya (*Stenocereus-Hylocereus*)[F]	Ponytail palm (*Beaucarnea* Lem.)[O]
Amaranth (*Amaranthus* L.)[B]	Echeveria (*Echeveria* DC.)[O]	Purple mombin (*Spondias*)[F]
Annatto (*Bixa* L.)[I]	Goatnut (*Simmondsia*)[B]	Purslane (*Portulaca* L.)[I]
Annona (*Annona* L.)[F]	Grape (*Vitis* L.)[F]	Quelite (*Chenopodium* L.)[I]
Avocado (*Persea* Mill.)[F]	Guava (*Psidium* L.)[F]	Sapotes (*Pouteria*)[F]
Bean (*Phaseolus* L.)[B]	Maize (*Zea mays* L.)[B]	Seepweed (*Suaeda*) Forssk. ex J. F. Gmel.)[I]
Bromeliad (*Tillandsia*)[O]	Mexican hawthorn (*Crataegus* L.)[F]	Squash (*Cucurbita* L.)[H]
Cacao (*Theobroma* L.)[F]	Nance (*Byrsonima Rich. ex Kunth*)[F]	Sunflower (*Helianthus* L.)[B]
Cactus (Cactaceae Juss.)[O]	Nopal (*Opuntia* L.)[F]	Sweet potato (*Ipomoea* L.)[H]
Cassava (*Manihot* Mill.)[I]	Orchids (Orchidaceae Juss.)[O]	Tigridia (*Tigridia Juss.*)[O]
Chayote (*Sechium* P. Browne)[H]	Papaya (*Carica* L.)[F]	Vanilla (*Vanilla* Mill.)[B]
Cotton (*Gossypium* L.)[B]	Pecan (*Carya* Nutt.)[F]	
Dahlia (*Dahlia* Cav.)[O]	Physic nut (*Jatropha*)[B]	

[B] Basic and Industrial, [F] Fruit trees, [H] Vegetables, [O] Ornamentals, [I] Emerging crops

3.3 Ex Situ Conservation

3.3.1 *Capacity of Ex Situ Conservation of Plant Genetic Resources in Mexico*

Córdova and Molina published in 2006 the second country report on plant genetic resources for food and agriculture. The third report is about to be released, but at the moment, the 2006 report is the latest official document containing country-wide statistics on ex situ conservation of plant genetic resources. The report indicated that nationwide, 276,945 germplasm accessions are under ex situ conservation. For orthodox seeds, there were 22 cold rooms with a total storage capacity of 2354 m^3, from which 1273 m^3 (54%) are used for the preservation of 54,945 accessions. However, only a few were operated under conditions that ensured long-term preservation (in cold rooms at less than 0 °C). Another 52,268 accessions were conserved by researchers at different institutions, in rooms with no controlled conditions (ambient temperature). About 69,931 accessions are held in working collections and also in rooms at ambient temperature. Córdova and Molina (2006) noted that under these storage conditions, many of these accessions could easily be lost. Regarding documentation of accessions, the authors mentioned that information records were not complete and, in some cases, even nonexistent. Documentation of germplasm was performed with field books, electronically, or both. Computer programs frequently used were Access, Excel, Word, Biotic, etc., with enough capacity to generate a database; however a comprehensive system is required to organize all the data at a national level (Córdova and Molina 2006).

More recently, to protect ex situ germplasm in Mexico, the SAGARPA, through the SNICS, implemented the National System of Plant Genetic Resources for Food and Agriculture (Corzo 2013), which integrates the Conservation Center Network (CCN). It is composed of 4 orthodox seed conservation centers, 3 recalcitrant seed conservation centers (in vivo), 19 working collections, 2 in vitro collections, and 26 community banks (Fig. 3.3, Table 3.3) (González-Santos et al. 2015).

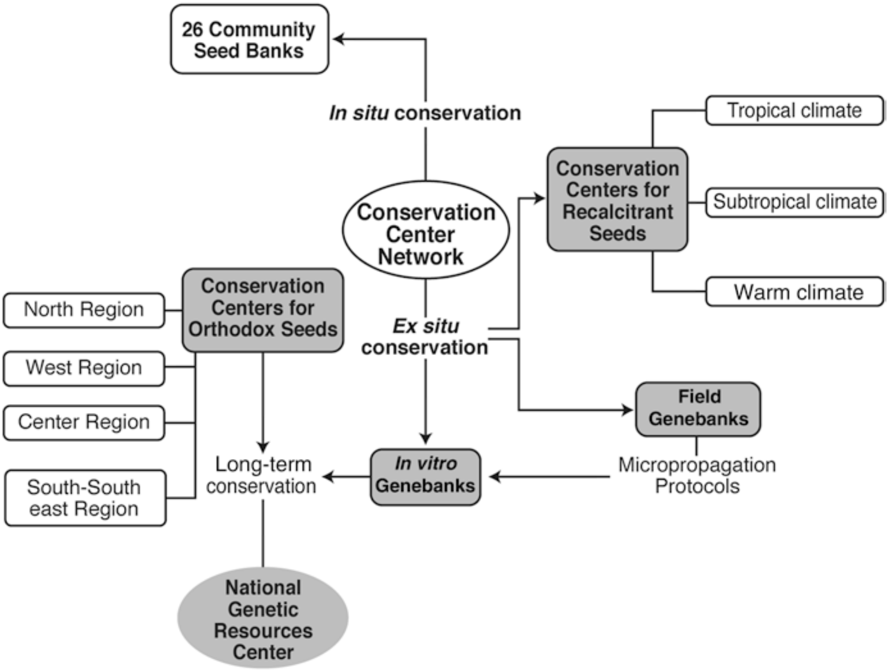

Fig. 3.3 Ex situ conservation strategy of plant genetic resources in Mexico, through the Conservation Center Network

Table 3.3 Number of accessions held at conservation centers (González-Santos 2016)

Conservation center	Genera	Species	Accessions
Orthodox seeds (UACh, UAAAN, UDG, ICAMEX)	292	1083	52,169 (83.2%)
Recalcitrant seeds (INIFAP, CICTAMEX, UACh)	46	147	2421 (3.9%)
Working collections (various)	248	703	7920 (12.6%)
In vitro collections (UGto, UV)	27	70	226 (0.4%)
Total			*62,736*

UACh Chapingo Autonomous University, *UAAAN* Antonio Narro Agrarian Autonomous University, *UDG* University of Guadalajara, *ICAMEX* Institute for Training Research and Development in Agriculture of the State of Mexico, *INIFAP* National Institute of Forestry Agriculture and Livestock Research, *CICTAMEX* Scientific and Technological Research Center of Avocado in the State of Mexico, *UGto* University of Guanajuato, *UV* University of Veracruz

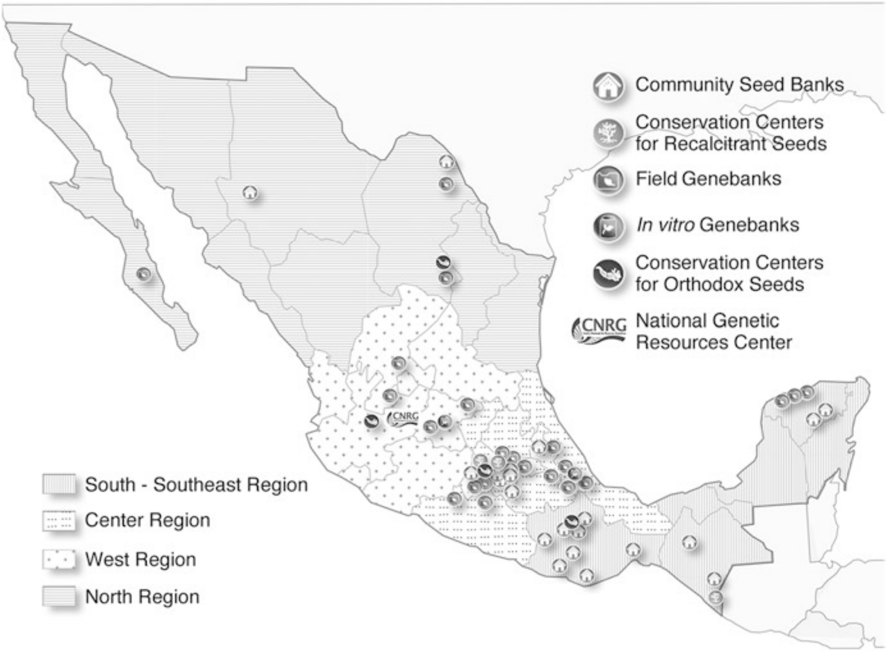

Fig. 3.4 Distribution of Conservation Center Network in Mexico

The strategy implemented for ex situ conservation divides the country into four regions, North, West, Center, and South-Southeast (Fig. 3.4), with the aim of reducing germplasm mobilization and saving expenses. The new collections are sent to the nearest region. The North Region Orthodox Seed Conservation Center is located at the UAAAN in Saltillo, Coahuila, with a capacity for 7000 accessions. Currently, it conserves 420 accessions of 6 different crops, mainly maize, sunflower, bean, and pepper. The West Region Orthodox Seed Conservation Center is located at the University of Guadalajara. It has a capacity of 11,000 accessions; currently, it preserves 9901 accessions of 14 different crops such as maize, husk tomato, bean, pepper, jojoba, potato, and amaranth, among others (González-Santos 2016).

The Conservation Center Network houses 62,736 accessions (51 families, 317 genera, and 1360 species) of plant germplasm from the main native crops of Mexico and their CWR (Table 3.4). The 62,736 accessions in the collection are composed of a set of 7195 accessions of maize germplasm also found in the International Maize and Wheat Improvement Center (CIMMYT); 5577 maize accessions originating from the National Institute of Forestry, Agriculture and Livestock Research (INIFAP); as well as 8262 accessions of historic collections of different species originating from Mexico that were repatriated from the USDA National Plant Germplasm System. The SNICS Networks also collected approximately 36,690 accessions from 2002 to 2013. The National Genetic Resources Center (CNRG) conserves a duplicate of the accessions repatriated from the United States, CIMMYT, and INIFAP, with a total of 17,846 accessions of 98 genera and 277 species, as a safety backup of these collections (González-Santos 2016).

The south-southeast Orthodox Seed Conservation Center is located in the Southern University Campus of the Chapingo Autonomous University (UACh) in Oaxaca. It has the capacity to store 5000 accessions and currently preserves 2500 accessions of crops such as beans, pepper, maize, amaranth, cotton, squash, and papaya, among others. Finally, the Center for Orthodox Seed Conservation Region Center is located at the main campus of the UACh, in Texcoco, State of Mexico. It has the capacity to store 50,000 accessions and currently preserves 16,792 accessions representing over 72 families, 214 genera, and 295 species of wild and cultivated species used for food, medicine, and fuel in Mexico. Six hundred accessions of maize and its CWR (teosinte and *Tripsacum*) are preserved in this seed bank, as well as accessions of common bean, scarlet runner bean, amaranth, husk tomato, pepper, dahlia, and marigold, among others (Molina et al. 2014, Universidad Autónoma Chapingo 2013).

Recalcitrant seed preservation centers are located at (1) Rosario Izapa Research Station, INIFAP, in Tapachula, Chiapas, where 87 accessions of crops such as cacao, marmalade-plum (*Pouteria sapota*), cassava, and others are preserved; (2) Coatepec Harinas, State of Mexico, the collection of the Salvador Sánchez Colín-CICTAMEX Foundation, which preserves 384 accessions of crops such as dragon fruit, grape, nance (*Byrsonima crassifolia* (L.) Kunth), cherimoya, avocado, and sweet potato, among others; and (3) UACh, in Texcoco, State of Mexico, where 414 accessions of crops such as Mexican hawthorn (*Crataegus mexicana* DC.), agave, cactus, and orchids are conserved (Molina et al. 2014).

3.3.2 Ex Situ Conservation of Endangered Species

The NOM-059-SEMARNAT-2010 (NOM-059) is the Mexican standard that lists all threatened native wild species (DOF 2015). In the list, there are 105 CWR and WUS species under various risk categories that are conserved by the CCN, represented by 964 accessions. Of importance are the genus *Echeveria* with 100% of the listed species collected (12) and the genus *Beaucarnea* (ponytail palm) with 7 out of 9 listed

Table 3.4 Number of genera, species, and accessions per family, conserved by the Conservation Center Network

Family	Genera	Species	Accessions	Family	Genera	Species	Accessions
Agavaceae Dumort.	7	80	628	Fabaceae Lindl.	32	94	8353
Alliacea Borkh.	1	1	2	Fouquieriaceae DC.	1	1	1
Amaranthaceae Juss.	5	18	1536	Iridaceae Juss.	1	27	184
Amaryllidaceae J. St.-Hil.	4	15	85	Juglandaceae DC.	2	4	54
Anacardiacea R. Br.	1	1	113	Lamiaceae Martinov	4	6	6
Annonaceae Juss.	1	4	547	Lauraceae Juss.	3	15	1501
Apiaceae Lindl.	6	6	34	Lythraceae J. St.-Hil.	1	17	94
Apocynaceae Juss.	1	2	2	Malpighiaceae Juss.	1	1	63
Araceae Juss.	1	1	2	Malvaceae Juss.	5	16	1221
Asparagaceae Juss.	1	8	98	Martyniaceae Horan.	1	1	1
Asteraceae Bercht. & J. Presl	24	102	4107	Myrtaceae Juss.	1	4	238
Begoniaceae C. Agardh	1	2	3	Onagraceae Juss.	1	1	1
Bixaceae Kunth	1	1	79	Orchidaceae Juss.	86	299	2171
Boraginaceae Juss.	1	1	1	Pedaliaceae R.Br.	1	1	60
Brassicaceae Burnett	4	8	24	Phytolaccaceae R.Br.	1	1	3
Bromeliaceae Juss.	9	63	911	Plantaginaceae Juss.	1	3	4
Cactaceae Juss.	33	172	3021	Poaceae Barnhart	38	77	24,300
Caricaceae Dumort.	1	1	456	Polygonaceae Juss	1	1	5
Chenopodiaceae Vent.	2	10	175	Portulacaceae Juss.	1	1	75
Cleomaceae Bercht. & J. Presl	1	1	1	Rhamnaceae Juss.	1	1	1
Convolvulaceae Juss.	1	19	230	Rosaceae Juss.	1	6	332

(continued)

Table 3.4 (continued)

Family	Genera	Species	Accessions	Family	Genera	Species	Accessions
Crassulaceae J. St.-Hil.	2	123	966	Sapotaceae Juss.	1	1	125
Cucurbitaceae Juss.	8	26	1279	Simmondsiaceae Tiegh.	1	1	237
Dioscoreaceae R. Br.	1	2	538	Solanaceae Juss.	7	71	7293
Ericaceae Juss.	1	1	13	Vitaceae Juss.	1	4	179
Euphorbiaceae Juss.	5	38	1383	*Total*	*317*	*1360*	*62,736*

Table 3.5 Species listed in the NOM-059-SEMARNAT-2010, collected and safeguarded at the Conservation Center Network

Network	Species in the NOM-059	Stored species	Accessions
Agave (*Agave* L.)	39	8	14
Bromeliad (Subfamily Tillandsioideae)	21	4	124
Cactus (Family Cactaceae Juss.)	270	49	234
Dahlia (*Dahlia* Cav.)	2	1	64
Dragon fruit (*Hylocereus* (A. Berger) Britton & Rose)	3	0	0
Echeveria (*Echeveria* DC.)	12	10	57
Hymenocallis (*Hymenocallis* Salisb.)	5	2	2
Maize (*Zea diploperennis* H.H. Iltis et al.)	2	1	2
Nopal (*Opuntia* Mill.)	3	0	0
Orchids (*Encyclia* Hook., *Prosthechea* Knowles & Westc., *Laelia* Lindl., *Rhynchostele* Rchb. f. and *Stanhopea J. Frost ex Hook*)	187	20	224
Physic nut (*Jatropha* L.)	1	0	0
Poinsettia (*Euphorbia* L.)	12	0	0
Ponytail palm (*Beaucarnea* Lem.)	9	7	94
Purple mombin (*Spondias* L.)	1	0	0
Tigridia (*Tigridia* Juss.)	6	2	3
Vanilla (*Vanilla* Mill.)	1	1	146
Total	*574*	*105*	*964*

species collected (77%). For other genera, the collection has gaps, for example, in the genus *Euphorbia* (poinsettia), none of the 12 listed species have been collected. Table 3.5 shows the species listed in the NOM-059 that are collected and stored by the CCN. In Table 3.6, the number of species and accessions that are housed by the CCN are presented.

In addition to conservation centers established as part of the SAGARPA-SNICS ex situ conservation strategy, there are other germplasm collections and gene banks in Mexico, which are summarized below (Molina and Córdova 2006).

Table 3.6 Number of crop wild relative accessions safeguarded by the Conservation Center Network

Crop (genus)	CWR species	Accessions	Crop (genus)	CWR species	Accessions
Agave (*Agave* L.)	64	148	Nance (*Byrsonima Rich. ex Kunth)F*	1	48
Amaranth (*Amaranthus* L.)	7	65	Nopal (*Opuntia* L.)	30	357
Annatto (*Bixa* L.)	1	1	Orchids	479	2975
Avocado (*Persea* Mill.)	7	38	Papaya (*Carica* L.)	1	138
Bean (*Phaseolus* L.)	26	884	Pecan (*Carya* L.)	3	19
Bromeliad (*Tillandsia* L.)	48	806	Pepper (*Capsicum* L.)	2	212
Cacao (*Theobroma* L.)	1	1	Physic nut (*Jatropha* L.)	5	63
Cactus (Cactaceae)	78	278	Pitaya (*Stenocereus* (A. Berger) Riccob.*)*	6	55
Cassava (*Manihot* Mill.)	1	42	Poinsettia (*Euphorbia* L.)	2	5
Chayote (*Sechium* P. Browne)	4	30	Ponytail palm (*Beaucarnea* Lem)	5	42
Cotton (*Gossypium* L.)	9	601	Potato (*Solanum* L.)	17	68
Dahlia (*Dahlia* Cav.)	17	105	Purple mombin (*Spondias* L.)	1	2
Dragon fruit (*Hylocereus* (A. Berger) Britton & Rose*)*	4	136	Purslane (*Portulaca* L.)	1	5
Echeveria (*Echeveria* DC.)	95	329	Quelite (*Chenopodium* L.)	19	32
Grape (*Vitis* L.)	1	86	Sapote (*Pouteria* Aubl.)	0	0
Guava (*Psidium* L.)	1	40	Seepweed (*Sauceda* Forssk. ex J. F. Gmel.)	5	122
Husk tomato (*Physalis* L.)	18	190	Squash (*Cucurbita* L.)	10	79
Hymenocallis (*Hymenocallis* Salisb.)	12	85	Sunflower (*Helianthus* L.)	8	191
Maize (*Zea* L.)	2	111	Sweet potato (*Ipomea* L.)	3	22
Marigold (*Tagetes* L.)	21	1355	Tigridia (*Tigridia* Juss.)	24	165
Mexican hawthorn (*Crataegus* L.)	0	0	Tomato (*Solanum* L.)	3	749
Mountain yam (*Dioscorea remotiflora* Kunth)	2	374	Vanilla (*Vanilla* Mill.)	4	40

3.3.3 Ex Situ Conservation of Plant Germplasm at INIFAP

In 2012 and 2013, the National Center for Genetic Resources (CNRG) of INIFAP carried out an analysis (unpublished data) in order to better understand the state of conservation and locations of collections preserved by INIFAP. The analysis showed that 78,066 accessions of orthodox agricultural seeds from 34 species of 17 genera were conserved in cold chambers or cold rooms. A total of 4786 accessions of 70 species of 45 genera were conserved ex situ in field collections. For example, the field collections of cocoa and *Jatropha* are conserved at the Rosario Izapa Research Station located in Tapachula, Chiapas. Agave species are conserved at the Valles Centrales Research Station located in Etla, Oaxaca, and the field collections of avocado are conserved at the Bajío Research Station located in Celaya, Guanajuato, and at the Uruapan Research Station located in Uruapan, Michoacan. Table 3.7 describes the INIFAP orthodox seed germplasm banks, where important collections of crops and CWR are preserved.

At the CNRG, 25,450 accessions are preserved, of which 24,294 are accessions of agricultural species such as maize, beans, nopal, amaranth, pepper, pumpkins, sunflower, sweet potato, ground cherry, tomato, potato, medicinal species, forage species (pastures and legumes), forest species and accessions of wild relatives of various crops such as teosinte, and wild beans. Moreover, 272 accessions of other native species such as vanilla, cacao, potato, chayote, husk tomato, pepper, tequila agave, and dragon fruit are conserved at the CNRG. A total of 4455 accessions of *Phaseolus* were repatriated from the International Center for Tropical Agriculture (CIAT), including 915 accessions of bean wild relatives, mainly *P. vulgaris* L., *P. acutifolius* A. Gray*, P. coccineus* L.*, P. lunatus* L.*, P grayanus* Wooton & Standl.*, P. magnilobatus* Freytag & Debouck*, P. microcarpus* Mart.*,* and *P. parvifolius* Freytag, among others.

The GRIN-Global genebank database platform is being implemented at the CNRG, so all the accessions have electronic passport data. In contrast, ex situ collections of several INIFAP Research Stations only have records in electronic spreadsheets, and the oldest research stations only have passport records in field books. The midterm plan of INIFAP (2019) is to have the passport data of all its collections uploaded to GRIN-Global.

3.3.4 Ex Situ Conservation of Crop Wild Relatives and Wild Utilized Species at CICY

The Yucatan Center for Scientific Research (CICY), located in Merida, Yucatan, has a germplasm seed bank linked to the field collections of the CICY Botanical Garden, where research is carried out with species having agroecological, medicinal, forestry, and food uses; most of them are native species. In addition, it has a collection of native maize of the Peninsula of Yucatan. Likewise, it also has a regional

Table 3.7 Germplasm banks where important collections of orthodox seed species are preserved in INIFAP (CNRG, unpublished data)

Seed bank	Conservation infrastructure	Location	Accessions
National Genetic Resources Center (CNRG)	2 cold chambers for long-term preservation	Tepatitlán de Morelos, Jalisco	25,450 (470 species)
Zacatecas Research Station	1 cold chamber for long-term preservation	Calera, Zacatecas	13,777 (maize, beans, other species)
Valle de México Research Station (CEVAMEX)	1 cold chamber for active and long-term conservation	Texcoco, State of Mexico	24,000 (maize, teosinte, other species)
Phaseolus spp. germplasm bank CEVAMEX	1 cold chamber for active and long-term conservation	Texcoco, State of Mexico	12,496 (*Phaseolus* spp. and *Phaseolus* wild relatives)
Valles Centrales Research Station	1 cold chamber for active and long-term conservation	Etla, Oaxaca	2370 (maize, pumpkin, cotton, beans)
Zacatepec Research Station	1 cold chamber for active conservation	Zacatepec, Morelos	4089 (tomato, amaranth, rice, sesame)

botanical garden with six types of collections organized by use and a herbarium (Koleff et al. 2016; CICY 2017). The CICY also has a germplasm collection of *Agave*, which is a working collection of vegetative material of wild and cultivated populations of agave. The collection includes wild specimens of *Agave angustifolia* Haw. from Guerrero, Jalisco, Oaxaca, Sonora, Veracruz, and the Yucatan Peninsula, as well as from Guatemala, and cultivated mescal agave from Jalisco and Oaxaca. The wild agaves of the Yucatan Peninsula include 3 ecotypes and 3 varieties recognized by farmers for the quality of their fiber, and those of Jalisco include 11 populations from the center and south of this state (Colunga 2004).

3.3.5 Ex Situ Conservation of Crop Wild Relatives and Wild Utilized Species at FES-Iztacala, UNAM

The seed bank of arid and semiarid zones of Mexico is located in the Faculty of Higher Education Iztacala (FES-I) of the National Autonomous University of Mexico (UNAM) located in Tlalnepantla, State of Mexico. This germplasm seed bank conserves seeds of threatened wild native species, either rare because they have local or very restricted distribution or are vulnerable due to the alteration of habitat and overexploitation. This seed bank currently has 3614 accessions, belonging to 125 families, 750 genera, and 1912 species. The best represented families are Asteraceae, Fabaceae and Cactaceae, which are among the 15 families with the highest number of native species in Mexico (Rodríguez-Arevalo et al. 2016).

Since February 2002, the Royal Botanical Gardens Kew (RBG Kew) of the United Kingdom and the FES-I have worked together on the conservation of seeds of endemic wild species of rare and threatened plants or wild relatives of economically important species from the arid and semiarid zones of Mexico through the "Useful Plants Project" (UPP), as part of the Millennium Seed Bank Project (Ulian et al. 2016).

The UPP collected seeds of 204 species of plants which are useful to the local communities of the valley of Tehuacán-Cuicatlán. These seeds are conserved in the FES-I Germplasm Seed Bank, and of the total collected, a duplicate of 134 species were sent to the Millennium Seed Bank in the United Kingdom. Regarding the use of the species conserved by the UPP project in the FES-I Germplasm Seed Bank, 16.2% of the species are used as fodder, 22.3% have a use in the environment, 18.7% have medicinal use, 15.7% are used as food, 13.7% are used for materials, 7.8% are used as fuel, 3.3% have a cultural use, 2.0% are used as poison and 0.2% are weeds (Rodríguez-Arevalo et al. 2016).

3.3.6 Ex Situ Conservation of Crop Wild Relatives at CIMMYT

Finally, there is the International Maize and Wheat Improvement Center (CIMMYT), located in Texcoco, State of Mexico, which is a part of the CGIAR. CIMMYT's goal is to increase the efficiency of sustainable maize and wheat production systems, to ensure global food security and reduce poverty (CIMMYT 1986). The bank has a storage capacity of 450,000 seed samples, and seed treatment and packaging facilities have been specially designed to support the global role of CIMMYT in the conservation and distribution of germplasm (CIMMYT 1986). The wheat collection consists of 140,000 samples from 100 countries. There are 28,000 maize seed samples conserved, including the largest collection of maize landraces in the world (CIMMYT 1986) as well as samples of maize wild relatives such as teosinte (Taba 1995) and *Tripsacum* (Berthaud et al. 1995).

3.3.7 Acquisition and Distribution of Germplasm

A legal framework for the exchange of ex situ germplasm has not yet been established in Mexico. The acquisition and exchange of germplasm occurs only within institutions, universities, and organizations, and international exchange represents only a small percentage of the total exchange of germplasm (and mainly acquisition from other countries and CGIAR centers by Mexico). The lack of a legal framework has been a serious limitation and has impeded exchange of germplasm in Mexico (Cuevas and Hernández 2006).

More recently, with the encouragement of SINAREFI, national institutions have started to define regulations for the exchange of germplasm and have developed

Material Transfer Agreements (MTAs) that set the conditions for access to ex situ collections and benefit sharing. As we already mentioned, Mexico is not a member of the ITPGRFA; therefore, bilateral agreements are required for international exchanges of germplasm (Cuevas and Hernández 2006; Gonzalez-Santos et al. 2015).

Even with the difficulties that exist in the country, due to unclear rules for the exchange of genetic resources at the international level and lack of participation in the ITPGRFA, it is possible to establish collaborative agreements such as that signed between the RBG Kew and FES-I. The collaboration between the two institutions was established under an access and benefit sharing agreement (ABSA), as part of the Millennium Seed Bank Project (MSBP) (Eastwood and Linington 2012). The "Useful Plant Project" (UPP) managed by Kew uses an applied scientific approach to conserve and sustainably use indigenous plants that are important for local rural communities in Mexico. The same approach has been used in other countries such as Botswana, Mali, Kenya, and South Africa, bringing together the RBG Kew staff and a wide range of national scientific institutions to assist local communities in addressing the environmental challenges that threaten their livelihoods through the conservation and sustainable use of indigenous plant species (Ulian et al. 2016).

3.3.8 Ex Situ Conservation of Globally Important Crops and Their Wild Relatives

This section summarizes the occurrence and conservation of major crops and their genetic resources originating from Mexico but used around the world. For further details refer to the individual crop chapters in this book.

3.3.8.1 Maize

There are two perennial, one diploid and one tetraploid, species of teosinte, the closest relatives of maize. The taxonomy of *Zea* proposed by Doebley and Iltis (1980) described four teosintes for Mexico: *Zea perennis* (Hitchc.) Reeves & Mangelsd. (found in Jalisco and Colima), *Zea diploperennis* (found in Jalisco), *Zea mays* subsp. *parviglumis* H.H. Iltis & Doebley (found in Nayarit, Jalisco, Michoacan, Guerrero, and Oaxaca), and *Zea mays* subsp. *mexicana* (Schrad.) H.H. Iltis (found in Chihuahua, Guanajuato, Jalisco, Michoacan, Mexico City, State of Mexico, Puebla, and Tlaxcala) (Sánchez 2011).

3.3.8.2 Bean

Two primary centers of diversity are recognized for common bean (*Phaseolus vulgaris*), the Mesoamerican and Andean centers. There are 70 species of *Phaseolus* reported for Mexico, including the wild and cultivated forms of the 5 domesticated species: common bean (*P. vulgaris*), scarlet runner bean (*P. coccineus*), lima bean (*P. lunatus*), tepary bean (*P. acutifolius*), and year bean (*P. dumosus*) (Lépiz-Idelfonso and Ramírez Delgadillo 2010).

3.3.8.3 Pepper

Mexico is a secondary center of diversity of peppers, so it is considered a strategic location for the conservation and use of these genetic resources. Five cultivated species (*Capsicum annum* L.*, C. chinense* Jacq.*, C. pubescens* Ruiz & Pav.*, C. frutescens* L., and *C. baccatum* L.) and about 25 wild and semi-cultivated species are distributed in Mexico. There are 3894 accessions of the 5 cultivated species, *C. rhomboideum* (Dunal) Kuntze and other *Capsicum* species preserved by the CCN (Vera-Sánchez et al. 2016).

3.3.8.4 Cotton

Mexico is the center of origin of cultivated cotton. There are 50 fully identified *Gossypium* species, 4 of which are commercially grown (*Gossypium arboretum* L., *G. barbadense* L., *G. herbaceum* L., and *G. hirsutum* L.); the remaining 46 are considered CWR (Pérez et al. 2012), and 11 out of the 13 wild species of the western hemisphere are endemic to the country (Pérez et al. 2016). More than 1000 accessions of 3 species are maintained in an active collection, in addition to the establishment of a working collection of 127 accessions of 8 species and the collection and ex situ conservation of 508 accessions of 10 species.

3.3.8.5 Potato

About 200 wild species of *Solanum,* section *Petota* Dumort, are distributed from southwestern United States to central Argentina and Chile. There are 28 species of wild potatoes in Mexico, where a secondary center of diversity is located in the central Mexican highlands (Spooner et al. 2004). The CCN conserves 26 of the wild species with a total of 951 accessions (Vera-Sánchez et al. 2016).

3.3.8.6 Tomato

Mexico and Peru are considered the two independent domestication centers of tomato, hence the great diversity in both countries and the extensive collection they possess of these genetic resources (mainly landraces and wild species). Cultivated and wild tomatoes are widely distributed in Mexico, mainly in the central and southern states (Lobato-Ortiz et al. 2012). The CCN conserves 1291 accessions of *Solanum lycopersicum* L., *S. pimpinellifolium* L., and *S. betaceum* Cav. (Lobato-Ortiz et al. 2012, Vera-Sánchez et al. 2016).

3.3.8.7 Avocado

The genus *Persea* has about 200 species, distributed in tropical and temperate zones of the world, except in Africa. In Mexico there are at least 20 species related to the common American avocado. In order to classify avocado diversity, they are grouped in races; three of them are recognized in the country: Mexican, Antillean, and Guatemalan (Barrientos-Priego 2010; Gutiérrez-Díez and Mayek-Pérez 2014). According to Ramírez-Galindo et al. (2017), 1501 accessions of 12 species were collected in Mexico. The collections are distributed mainly along the Trans-Mexican volcanic belt, for their conservation by CCN.

3.3.9 Ex Situ Conservation in Arboreta, Botanical Gardens, and Ethnobotanical Gardens

Besides ex situ conservation of genetic resources in gene banks, arboreta and botanical gardens also conserve diversity and study behavior and adaptation to assess sustainable use of plant diversity in Mexico. Importantly, these institutes disseminate information on biodiversity conservation and sustainable use of plants to students and the general population.

In Mexico there are 57 arboreta and botanical gardens distributed in 26 of the 32 states. Thirty belong to the Mexican Association of Botanical Gardens (Caballero 2012) (Fig. 3.5). Only three of the botanical gardens are considered as ethnobotanical (i.e., they link wild and semidomesticated species to human uses). Arboreta and botanical gardens are mainly private entities, belonging to universities or civil organizations, but most receive funds from federal or state governments for maintenance, research, and dissemination activities. These include collections of native species and other species that are within the NOM-059 (FAO 2011; DOF 2015). Additionally, SEMARNAT has registered 418 nurseries and 73 botanical gardens as Wildlife Conservation Management Units (UMAs), which are distributed in all states of Mexico; however, UMAs do not indicate which species are at risk or participate in species recovery programs (SEMARNAT 2008).

3.3.9.1 Species Preserved in Botanical Gardens

The species conserved in arboreta and botanical gardens are reported by the Mexican Association of Botanical Gardens (AMJB). Although 51 official botanical gardens have been registered, only 37 botanical gardens are active and were recorded by the AMJB during the period 2000–2006 (Rodríguez-Acosta 2000). In total, 19 Mexican botanical gardens shelter a total of 4826 species, of which 441 species are included

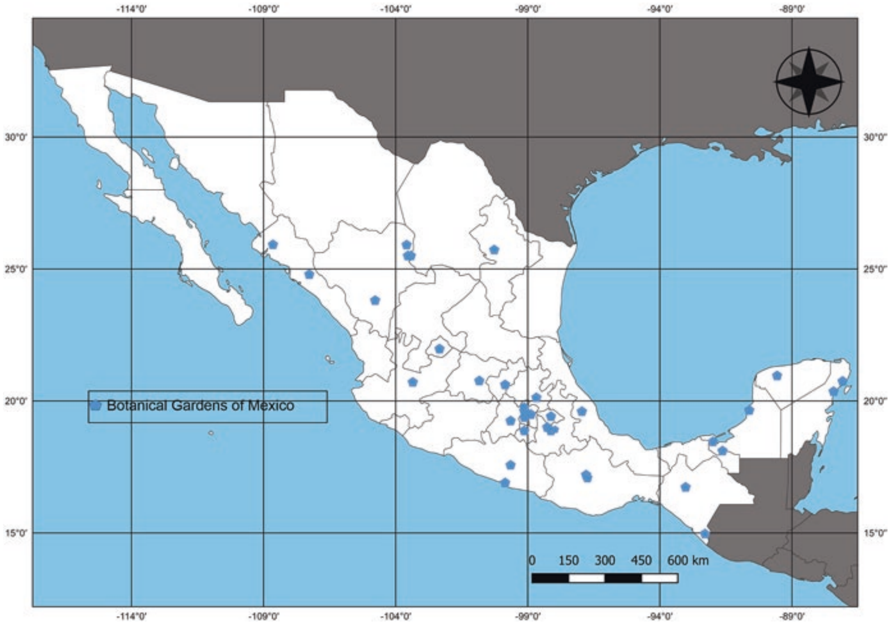

Fig. 3.5 Locations of botanical gardens, using data from CONABIO 2009

in the NOM-059; 2 are probably extinct in the wild, 92 are at risk of extinction, 167 are threatened, and 180 species are subject to special protection (Table 3.8) (Caballero 2012).

3.3.9.2 Policies and Regulations for Acquisition and Distribution of Germplasm in Arboreta and Botanical Gardens

The procedures for transfer of genetic material of protected species housed in botanical gardens, arboreta, nurseries, orchards, etc., must be carried out under the General Directorate of Wildlife of SEMARNAT, through forest transport documentation, under the Regulations of the General Law of Wildlife and/or paper vouchers issued by landowners, based on the provisions of the General Law on Sustainable Forest Development and its Regulations. The specific procedures can be found at http://tramites.semarnat.gob.mx/index.php/vida-silvestre/aprovechamiento/281-semarnat-08-031-a-informe-anual-de-actividades (accessed Apr 2017).

To promote the conservation of species, projects have been developed in Mexico with federal and state governments financing projects through 6-year programs. During the last 10 years, the federal government through CONAFOR has channeled economic resources for the protection and promotion of forest resources in arboreta and botanical gardens.

Table 3.8 Contribution of 19 Mexican botanical gardens to the protection of species listed in the NOM-059-SEMARNAT-2010

Botanical garden	Extinct in the wild	Endangered	Threatened	Under special protection	Total
IB-UNAM	2	39	95	130	266
El Charco del Ingenio	1	28	81	101	211
FESC-UNAM	1	19	50	67	137
Clavijero	0	50	37	19	106
Cadereyta	0	9	30	40	79
Etnobotánico de Oaxaca	1	24	22	28	75
Faustino Miranda	0	13	20	8	41
Culiacán	0	11	12	9	32
Cassiano Conzatti	1	5	9	14	29
CICY	0	3	18	8	29
INAH-Morelos	0	7	13	7	27
BUAP	1	7	9	6	23
Africam Safari	1	6	9	6	22
Ecosur	0	1	13	4	18
Xochitla	0	3	6	5	14
Franscisco Peláez	0	3	3	6	12
J. Rzedowski	0	1	4	3	8
Los Mochis	0	2	2	3	7
UAG	0	1	3	0	4
Total	2	92	167	180	441
Total in the NOM-059	6	183	340	456	985
Percentage	33.30%	50.27%	49.10%	47.50%	44.80%

The number of species in each risk category corresponds to the total in the NOM-059. Taken from Caballero (2012)

3.4 In Situ Conservation

3.4.1 In Situ Conservation of Maize Wild Relatives

The discovery in the mid-1970s of the wild maize – the endemic perennial teosinte *Zea diploperennis* – in its natural habitat in Jalisco, western Mexico, led to the establishment of the Sierra de Manantlán Biosphere Reserve in 1987. The in situ conservation of this species has been the central factor for the designation of this area, which also contains the teosintes *Z. perennis* and *Z. mays* subsp. *parviglumis*. This preservation of an entire ecosystem represents "the first and most spectacular story for *in situ* conservation of any wild relative of a crop plant" (Wilkes 1993).

Rather than aspire to pristine “nature” conservation (a concept that is increasingly criticized), the reserve incorporates local people’s land use and management. This is important for maize wild relatives, which have evolved in human-disturbed habitats and appear to require the continued cultivation of maize landraces (as well as grazing pressure) in order to thrive (Benz 1988).

3.4.2 In Situ Conservation of Cotton Wild Relatives

In Mexico there is not an in situ conservation program of cotton wild relatives in protected natural areas, only some studies in order to verify the current state of in situ conservation of wild cotton. In 2002 and 2003, Ulloa et al. (2006) carried out a survey on the status of cotton genetic resources in Mexico. Sixty years after the first in-depth studies of *Gossypium* in Mexico, they found that increasing human population, modernization, and urbanization have severely reduced the survival of *G. hirsutum* landraces. They located populations of seven known species, *G. aridum* (Rose & Standl) Skovst., *G. barbadense, G. gossypioides* (Ulbr.) Standl., *G. hirsutum, G. laxum* L. LI. Phillips, *G. lobatum* Gentry, and *G. schwendimanii* Fryxell & S.D. Koch, and one undescribed wild diploid *Gossypium* taxon during the survey. The study showed that the in situ conservation of some of these species is threatened due to the disturbance of the systems in which they live.

Furthermore, Ulloa et al. (2006) found that in G. *hirsutum*, the diversity that remains in situ is limited to feral plants that occur opportunistically in waste areas and as occasional home garden plants maintained as a novelty by rural peoples or village residents. *G. barbadense* is not generally thought to be significant in Mexico and in the study no wild plants were seen. On the other hand, *G. aridum* is the most widely distributed, occurring from Sinaloa to Oaxaca. In addition, this species is very diverse and does not appear to be threatened. In contrast, the in situ status of *G. laxum* is not fully known because the extent of its distribution range is unknown.

The distribution of the most recently described *G. schwendimanii* is unknown. *G. gossypioides* was encountered only in Oaxaca, and the explorations did not provide any new information on its distribution. The distribution of *G. trilobum* (DC.) Skovst. is generally limited to moderately high altitude in western Mexico, and although the status of *G. trilobum* in remote areas is unknown, the results of these surveys indicated that the distribution of this species has been severely eroded by agricultural and human-population pressures on its habitat (Ulloa et al. 2006). In contrast Pérez et al. (2016) reported that in situ conservation of semidomesticated *G. hirsutum* is carried out by indigenous ethnic groups. In the northwest of Nayarit, on the Mountains of the Sierra Madre, several Huichol native groups preserve in situ wild cotton plants of *G. hirsutum* that they use in religious ceremonies.

3.4.3 In Situ Conservation of Potato Wild Relatives

In Mexico, areas have been identified where two wild potato species (*Solanum cardiophyllum* Lindl. and *S. ehrenbergii* (Bitter) Rydb.) are being conserved in situ to be used for self-consumption and sale. *S. cardiophyllum* is collected in the states of Aguascalientes, Hidalgo, Jalisco, State of Mexico, Michoacan, Morelos, Oaxaca, Puebla, Queretaro, Sinaloa, Zacatecas, and Mexico City. *S. ehrenbergii* grows in Aguascalientes, Guanajuato, Hidalgo, Jalisco, State of Mexico, Michoacan, Nayarit, Puebla, Queretaro, San Luis Potosi, Zacatecas, and Mexico City. Their tubers are harvested for self-consumption and sale. Plants are tolerated among maize and bean cultivation fields (Vera-Sánchez et al. 2016).

In the highlands of San Luis Potosi and Zacatecas, tubers of *S. cardiophyllum* and *S. ehrenbergii* are sold for human consumption. In the community of San Ignacio in Villa Hidalgo, Jalisco, both species have been cultivated during the last 8 years, from tubers of wild populations (Vera-Sánchez et al. 2016).

3.4.4 In Situ Conservation of Husk Tomato Wild Relatives

The husk tomato network proposed a model of in situ conservation of wild populations and native varieties of husk tomato (*Physalis* spp.) in Jalisco. It is based on the production systems observed in Yahualica, Cuquío, and Techaluta. In Yahualica, conservation of *P. philadelphica* Lam. is aimed under various modalities: (1) tolerated within the "milpa" system, (2) promoted among cultivated fields, (3) cultivated in association, and (4) monoculture. In Cuquío, the model is applied for the conservation of *P. angulata* L. In Techaluta, *P. angulata* is also conserved following two production systems: (1) tolerated in rain-fed maize fields and (2) residual moisture in chickpea fields (Vera-Sánchez et al. 2016).

3.4.5 In Situ Conservation of Wild Utilized Species

Other important activities for in situ conservation of CWR and WUS are the implementation of Wildlife Conservation Management Units (UMAs), a protected area management model recently implemented by the Mexican government, and Natural Protected Areas (NPAs) (CONABIO 2011). In order to strengthen the Protected Areas State Systems in the country, the National Commission of Natural Protected Areas (CONANP) and the state governments started in 2009 a process of communication, coordination, and training focused on improving the capacities of the states for the management and administration of the NPAs, and in 2010 the National Network of Natural Protected Area State Systems was established.

Most Mexican NPAs are perceived as multipurpose areas where economic activities are limited because there is not a long-term program for the sustainable use of

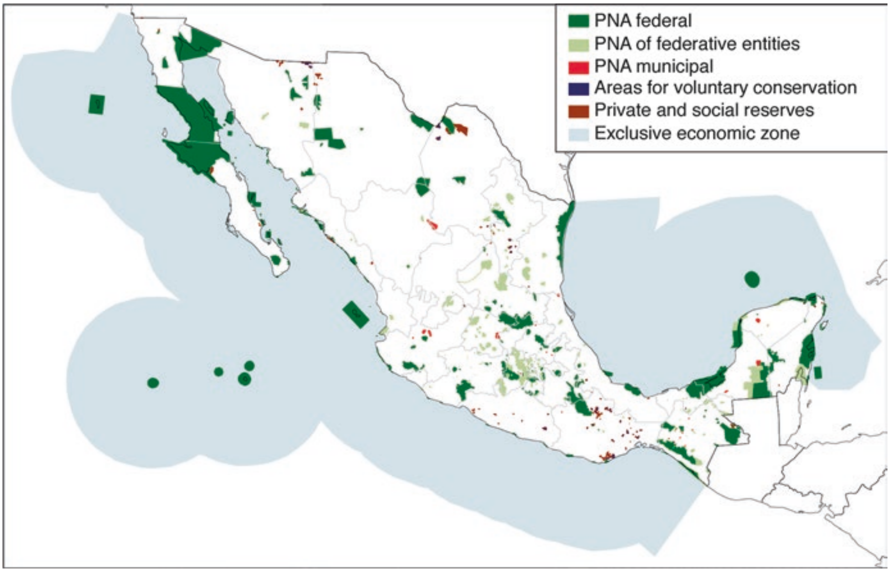

Fig. 3.6 Natural Protected Areas in Mexico for conservation, sustainable use, and restoration of natural resources (Bezaury-Creel and Gutiérrez 2009)

natural resources. In the Mexican territory, there are about 900 NPAs, federal, state, or municipal, as well as voluntary conservation areas and private and community NPAs (95 federal, 127 state, 11 municipal, 4 from Mexico City, 20 certified, and 22 private and social) (Fig. 3.6).

In Mexico, many attractive ornamental species of cacti are being placed at risk because of habitat destruction or change in land use, uncontrolled tourism, and poaching, which make their often small populations vulnerable to extinction. A number of actions that favor in situ conservation are being taken at federal, state, and local levels, both in Mexico and in the United States. Clear examples are the creation of NPAs: the Tehuacán-Cuicatlán Biosphere Reserve near Mexico City, the Pinacate and Altar Biosphere Reserve in the US-Mexico border area, the Vizcaino Biosphere Reserve in Baja California Sur, and the Flora Protection Areas in neighboring Texas. Most cactus species occur outside the protected areas, and these may be managed in UMAs. The UMAs attempt to bring together economic development of local communities and the conservation of the environment (Garpow 2001; Heywood and Dulloo 2005).

3.5 General Assessment of Conservation of Native Wild Plant Genetic Resources

CWR are subjected to several threats, such as intensive farming, land use change, climate change, nitrogen deposition, and biotic exchange (e.g., alien invasive species), among others. From those threats, climate change seems to be the most worrying. In the Mesoamerican region, dominated by subtropical and tropical climates, the prospects of CWR of important crops seem more secure under climate change, compared to other regions. Thomas et al. (2016) calculated net changes in the environmental suitability of ten Mesoamerican crops and their CWR from 2015 to 2050 and found a negative net change for the majority of the crops under study but opposite results when they analyzed CWR (Table 3.9). Figure 3.7a depicts the net change of suitability of the ten crops together, and Figure 3.7b depicts the net change of suitability for the respective CWR. The scenarios presented were modeled considering a condition of unrestricted dispersal of the species, although the prevalence or disappearance of the crops or their CWR may vary due to human intervention, i.e., species-assisted migration, limiting their dispersion. It is also pertinent to mention that this prediction does not take into account other threats to crops and CWR.

Table 3.9 Predicted gains, losses, and net changes in suitable areas (in millions of hectares), from 2015 to the 2050s of crop species and their CWR grouped together per genepool, as well as for all genepools together, based on environmental niche modeling

	Crop species				Crop wild relatives			
Genepool	Gain	Loss	Net change		Gain	Loss	Net change	
Cucurbita	123.4	65.9	+57.5	⬆	158.5	12.5	+146.0	⬆
Amaranthus	24.7	12.7	+12.0	⬆	108.0	63.9	+44.1	⬆
Capsicum	67.1	43.6	+23.5	⬆	5.7	12.8	–7.1	⬇
Carica	7.2	40.4	–33.2	⬇	1.9	13.9	–11.9	⬇
Tripsacum	12.5	34.9	–22.4	⬇	89.6	29.7	+59.9	⬆
Ipomoea	1.9	35.1	–33.2	⬇	223.5	18.9	+204.6	⬆
Phaseolus	40.4	72.7	–32.3	⬇	198.6	26.9	+171.7	⬆
Manihot	4.0	37.9	–33.9	⬇	242.0	1.0	+241.0	⬆
Persea	4.2	46.4	–42.3	⬇	207.0	5.4	+201.6	⬆
Zea	1.7	46.7	–44.9	⬇	89.3	22.2	+67.1	⬆
Mesoamerica	138.3	141.3	–3.1		241.1	10.5	+230.5	

From Thomas et al. (2016), with permission of the author

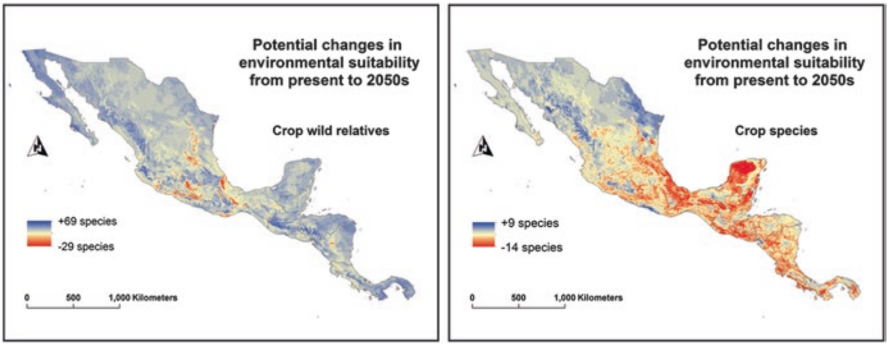

Fig. 3.7 (**a**) Potential changes in environmental suitability of crop species from present to the 2050s. (**b**) Potential changes in environmental suitability of crop wild relatives from present to the 2050s. (With permission from author (Thomas et al. 2016))

From the above information, we cannot infer that the presence of Mesoamerican CWR is guaranteed in the future. Their subsistence not only depends on climatic conditions but on many other factors, most of them human-driven. Factors, such as agricultural industrialization, globalization of markets, migration, local policies, cultural shifts from traditional to industrial systems, deforestation, and subsequent fragmentation of habitats, are a specific menace to Mesoamerican crops and CWR biodiversity (Harvey et al. 2008). It is undebatable that the Mesoamerican center of biodiversity, a hotspot of high biodiversity for domesticated crops and CWR, is threatened and that inaction will lead irreparably to significant loses of genetic resources that are valuable for the food security of the region and the entire world. Prompt action must be taken to mitigate the forces threatening the permanence of domesticated crops and their CWR in Mexico. Measures also need to be applied to the 600–700 undomesticated species that are being utilized directly from the wild by many indigenous people. Many of these species are CWR, and many of them are generically known as NUS (neglected, underutilized species).

Considering that Mexico is a mega-diverse country, with a great diversity of vascular plants, including CWR and WUS, the conservation of plant genetic resources is a task of the highest importance. As mentioned before, the status of conservation of CWR and WUS in terms of proportion collected out of the existing diversity is appallingly low. Mexico is not alone in this regard (Castañeda-Álvarez et al. 2016). According to Maxted and Kell (2009), the first step to address the conservation of CWR and WUS, is the development of a reliable inventory of species. Once an inventory is developed, a given country can define existing diversity, identify gaps in holdings of ex situ and in situ collections, develop acquisition plans, and prioritize efforts to characterize, document, and develop innovative conservation protocols. Even though the level of conservation of CWR and WUS in Mexico is generally low, important conservation efforts are occurring and are worth mentioning: the SNICS-SINAREFI Conservation Center Network; the "Useful Plants Project" based on the successful collaboration between the FES-I, UNAM, and the Millennium Seed Bank of the Royal Botanical Gardens Kew; the CIMMYT collections of maize CWR; the tropical tree collections of CICY; the collections at INIFAP research stations and the National Genetic Resources Center; the network of arboreta, botanical, and ethnobotanical gardens; and the federal, state, and municipal network of UMAs and NPAs.

3.6 Challenges and Opportunities to Conserve Wild Plant Genetic Resources

The challenge is to have a strong nationwide conservation system that can protect the diversity and richness of CWR and WUS in Mexico. Activities to achieve this would be:

1. To have a reliable inventory of CWR and WUS. This can be achieved by using the many documents and electronic information resources related to this subject,

available at several sources, such as SAGARPA through the SNICS, with the origin of the SINAREFI, and CONABIO programs and through several institutions that focus on this topic, including INIFAP, CP, UACh, and UDG, among others.
2. To perform a gap analysis of the most important CWR and WUS and, as much as possible, fill these gaps by acquiring new germplasm on collection trips.
3. To assure the financial support for the maintenance of ex situ collections, under all its forms of conservation.
4. To promote public policies for the support of in situ conservation of germplasm, such as generating incentive mechanisms to encourage genetic resource custodians to continuing their preservation activities, or developing mechanisms similar to the carbon bonus payment.
5. To promote the development of a more robust network of arboreta and botanical gardens and encourage managers of botanical gardens to broaden scope to ethnobotanical gardens.
6. To reinforce the concept of CWR and WUS and their importance at the UMAs and NPAs networks.

All these actions require the concurrence of all actors, including federal, state, and local governments, civil organizations, land owners, breeders, seed companies, universities, and research institutes.

Although the challenges are great, there are areas of opportunity worth mentioning:

1. The environmental and production sectors in Mexico are entering into a new integration era, where producing food supplies for ever-growing demand has the same priority as environmental conservation in sustainable systems.
2. The project safeguarding Mesoamerican crop wild relatives funded by the Darwin initiative and carried out by institutions, organizations, and universities of El Salvador, Guatemala, Honduras, Mexico, and the United Kingdom is aiming to generate capacity building for defining conservation actions for CWR, identifying areas of in situ conservation of threatened and vulnerable CWR, and conserving important Mesoamerican CWR species ex situ in national seed banks. The project will include phases of identification of CWRs, risk assessment, definition of conservation areas, and development of national plans. This activity will improve significantly the knowledge and status of conservation of CWR in Mexico and will tighten the collaboration with Mesoamerican countries with which we share diversity, culture, and objectives.
3. We recently prepared the first country report on biodiversity for food and agriculture, and a world report is under preparation. This FAO initiative will strengthen the concept of biodiversity mainstreaming with a direct impact on the conservation of ecosystems and hence CWR and WUS.
4. The president of Mexico decreed at the COP 13 in December, 2016, the greatest addition of natural protected areas in the history of Mexico. With this addition, the government of Mexico is committed to protect 910,000 Km^2 of Mexican territory, almost half of the country. With this action, Mexico takes an enormous

step toward the protection of wild plant species, including CWR and WUS. It is important to note that at the same time, this action represents a huge challenge, as more human, economic, and material resources will be required for the adequate management of the new protected areas.

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Chapter 4
Conservation of Crop Wild Relatives in the USA

Karen A. Williams and Stephanie L. Greene

Abstract Crop wild relatives (CWR) are found throughout the USA, with a high concentration in the eastern part of the country. These wild plants include the ancestors of crops domesticated within the borders of the country, such as sunflower, pecan, blueberry, cranberry, and squash, as well as the relatives of crops domesticated elsewhere. This chapter presents an overview of some of the CWR found in the USA and the status and potential for improved conservation through both ex situ and in situ approaches. The largest collection of CWR germplasm from the USA is the National Plant Germplasm System, managed by the US Department of Agriculture, Agricultural Research Service (USDA-ARS). Other germplasm conservation networks, including the Bureau of Land Management-led Seeds of Success, the Center for Plant Conservation's Collection of Endangered Plants, and the American Public Gardens Association/US National Arboretum-coordinated Plant Collections Network, support collection and conservation of plants that include CWR. Active sampling of CWR for ex situ conservation is ongoing, especially for certain crop groups, such as potato, sunflower, and small fruits. In situ conservation of CWR is mostly passive, involving protected areas that were established for other objectives. Ample opportunities exist to fill gaps in ex situ collections and to more deliberately conserve CWR on public lands, particularly by making better use of available sources of information, harnessing existing frameworks, and developing new partnerships.

Keywords Crop wild relatives · Wild utilized species · Ex situ conservation · In situ conservation · National Plant Germplasm System · United States

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K. A. Williams (✉)
USDA, Agricultural Research Service, Beltsville Agricultural Research Center, National Germplasm Resources Laboratory, Beltsville, MD, USA
e-mail: karen.williams@ars.usda.gov

S. L. Greene
USDA, Agricultural Research Service, Center for Agricultural Resources Research, National Laboratory for Genetic Resources Preservation, Fort Collins, CO, USA
e-mail: stephanie.greene@ars.usda.gov

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4.1 Overview of Important CWR and Wild Utilized Species (WUS) in the USA

Crop wild relatives in the USA include the wild progenitors of crops that were domesticated in the country, as well as other native and naturalized species closely related to crops domesticated elsewhere. Although the eight main geographic centers of origin of cultivated crops identified by Nikolai Vavilov in the 1920s and 1930s did not include the USA, more recent studies (Price 2016; Smith 2006) have added the eastern USA to the list of independent centers of crop domestication. At least four crops were domesticated in this region hundreds of years before maize, beans, and other crops were introduced from the south (Smith and Yarnell 2009). Two of these crops are widely grown today – the sunflower (*Helianthus annuus L.*) and the "ovifera" squash (*Cucurbita pepo* subsp. *ovifera (L.) D. S. Decker*) that includes some summer squashes and acorn squash (Smith 2006). As for the other two crops, chenopod (*Chenopodium berlandieri* Moq. subsp nuttalliae (Saff.) H.D. Wilson & Heiser) is today cultivated as a pseudocereal and leafy vegetable in Mexico, while marshelder (*Iva annua* L. var. *macrocarpa* (S.F. Blake) R.C. Jacks., nom. inq.) has been abandoned as a crop. At least three other plants in the eastern USA were potential crops that were intentionally planted and harvested: erect knotweed (*Polygonum erectum* L.), little barley (*Hordeum pusillum* Nutt.), and maygrass (*Phalaris caroliniana* Walter) (Smith and Yarnell 2009).

The southwestern (SW) USA also played a role in the domestication of some crops. Sonoran panic grass (*Panicum hirticaule* J. Presl var. *hirticaule*) seems to have been domesticated at least partly in the region (Nabhan and de Wet 1984). Domestication of amaranth (*Amaranthus hybridus* L.) and tepary bean (*Phaseolus acutifolius* A. Gray var. *acutifolius*) may have occurred partially there. Devil's claw (*Proboscidea parviflora* (Wooton) Wooton and Standl. subsp. *parviflora*), used for basketry, food, and medicine, is believed to have been domesticated in the area in historic times (Bretting and Nabhan 1986).

Other crops more recently developed within the current borders of the US post-European contact include blueberry, cranberry, wildrice, muscadine grape, pecan, raspberries, blackberries, and pawpaw. In addition, a number of wild relatives of crops domesticated in Mesoamerica, such as beans, cotton, maize, pepper, and pepo pumpkins and squashes, are native in the USA as well as in Mesoamerica. Many taxa in the secondary or tertiary genepools of crops domesticated in more distant lands, such as quinoa, hops, potato, and stone fruits, occur in the country. There are also forage and turf grasses, ornamentals, medicinal plants, industrial plants, and plants with potential as new crops.

Despite the emphasis placed over the past century on the importance of imported plants to agricultural development in the USA (Hodge and Erlanson 1956; National Plant Genetic Resources Board 1984), the presence and value of CWR and WUS native to the USA have been recognized for decades. The significance of native genetic resources for specific crops, including pecan (Heiges 1896), sunflower (Heiser 1969), blackberries and raspberries (Darrow 1937), and grapes (Munson 1909), has been elaborated in scientific treatments. Native CWR in the SW USA and their role in crop domestication and diversification have been described (Carter 1945; Nabhan 1990b). Also, the potential for use of CWR from the USA for crop improvement has been recognized in publications dating back many years (Beard 1977; Prescott-Allen and Prescott-Allen 1986).

Another significant development in the history of native CWR was the establishment of national genebanks wholly or partially dedicated to native crops and wild plants related to them. The New Crops Committee that represented the directors of agricultural experiment stations developed plans for four plant introduction stations in 1947. The plans included as one of the primary functions of the stations the "evaluation, cataloging and preservation of native plant materials or plants presently available in the USA that have not been adequately tested for industrial or agricultural use" (Burgess 1971). Each of these stations now includes native CWR and other native plants in their holdings. Some of the other repositories that were established beginning in 1980 to conserve clonally propagated, horticultural fruit and nut crops have a large component of native CWR in their collections (Postman et al. 2006). The National Clonal Germplasm Repository for Pecans and Hickories (est. 1984) was dedicated to native species, and the National Clonal Germplasm Repository in Corvallis, Oregon (est. 1980), has a significant representation of native species. Information on holdings, as well as taxonomy, common names, and geographical distributions of native CWR in the USA, has been available in the Germplasm Resources Information Network (GRIN, now GRIN-Global; https://www.ars-grin.gov/npgs/index.html) database since its inception.

Finally, over 20 years ago, a preliminary checklist of the native CWR was developed for the US Country Report (FAO 1995) produced for the United Nations' Food and Agriculture Organization (FAO)'s First Report of the State of the World's Plant Genetic Resources for Food and Agriculture. The checklist included all taxa in the genera of important crop species without consideration of their genetic relatedness.

Despite earlier progress, it was not until 2013 that the first attempt at a comprehensive listing of the CWR of the USA (Khoury et al. 2013) was published. The national inventory listed 2500 taxa of CWR representing 1905 species from 160 genera and 56 plant families in the USA. Sixty percent of the CWR species are related to food crops, particularly fruits, such as strawberry (*Fragaria* x *ananassa* Duchesne ex Rozier), blueberry and cranberry (various *Vaccinium* L. species), stone fruits (various *Prunus* L. species), and grape (*Vitis vinifera* L.). Native crop wild relatives were also identified for other important food crops including bean (various *Phaseolus* L. species), pecan (*Carya illinoinensis* (Wangenh.) K. Koch), peppers (various *Capsicum* L. species), sweet potato (*Ipomoea batatas* (L.) Lam.), quinoa (*Chenopodium quinoa* Willd.), squashes/pumpkins (various *Cucurbita* L. species), and sunflower (*Helianthus annuus*). Twenty-eight percent of the CWR species belong to either the family Fabaceae or the family Poaceae and are related to forage and fodder crops. The remainder of the CWR taxa identified are related to crops with a variety of uses, including medicinal, ornamental, material, and industrial. In addition to CWR, the inventory identified 2100 WUS, representing 833 genera and

182 families that are used directly as food, forage, medicine, and ornamentals and for environmental restoration. Of the top three use categories for WUS, 38% of the taxa are used for ornamental purposes, 35% are used for restoration, and 12% are used medicinally. Seventy-two of the total taxa in the inventory are listed as endangered or threatened under the US Endangered Species Act, including *Cucurbita okeechobeensis* (Small) L. H. Bailey (relative of the pepo and ovifera squashes and pumpkins and butternut squash) and *Zizania texana* Hitchc. (relative of wildrice).

Khoury et al. (2013) prioritized the taxa in the national inventory of CWR based on perceived value to contribute to breeding programs of major, minor, and nonfood crops, using the genepool and taxon group concepts of Wiersema and León (2016) and the Harlan and de Wet Crop Wild Relative Inventory (Vincent et al. 2012). The highest priority (P1) CWR taxa were defined as taxa related to important food crops and were further categorized into P1A taxa (native and closely related [i.e., genepools 1 or 2] or identified by researchers as potentially useful for breeding) and P1B taxa (either nonnative or native but more distantly related to important food species). Approximately 821 taxa were categorized as P1, with about 285 native taxa prioritized as P1A. Prioritization of taxa in the inventory provides the basis for future development of a conservation strategy for CWR in the USA.

4.1.1 Highlights of Genetic Resources in the USA

Decades ago, Prescott-Allen and Prescott-Allen (1986, 1990) brought attention to the contribution of CWR, including several native to the USA, to the country's economy. The search for sources of disease and pest resistance for crop improvement inspired much of the early use of CWR (Prescott-Allen and Prescott-Allen 1986). The first successful examples of the use of native CWR for disease and pest resistance, which occurred even before development of the science of genetics, were the uses of North American *Vitis* L. species to save the European wine industry. After phylloxera (*Daktulosphaira vitifoliae* (Fitch)), a relative of the aphid that is native to the eastern and southern USA, was unintentionally introduced into Europe in the mid-to-late 1800s, it attacked the roots of the nonresistant European *Vitis vinifera* grapes, weakening and finally killing the vines. The solution to this devastation was found mainly in the use of North American species *Vitis riparia* Michx., *V. rupestris* Scheele, and others to produce resistant rootstocks, a use that continues to this day (Gale 2002). Following are brief descriptions of some of the outstanding examples of crop genetic resources native to the USA and uses made of them.

4.1.1.1 Sunflower

Sunflower (*Helianthus annuus*), the most economically valuable crop native to the USA, has its center of origin in the eastern Central USA, where it was domesticated by native Americans between 5000 and 3800 years ago (Smith 2014; Blackman et al. 2011). It became an important oil seed crop in Russia, and improved varieties were introduced back into North America in the 1920s–1940s (Seiler et al. 2017). Crop wild relatives of sunflower have been used extensively in improvement efforts (Tyack and Dempewolf 2015; Hunter and Heywood 2011), with the use of cytoplasmic male sterility from *H. petiolaris* Nutt. and restorer genes from wild *H. annuus* and *H. petiolaris* being widely regarded as the CWR traits of greatest value (Seiler et al. 2017). In total, the sunflower CWR have contributed at least seven traits, mainly pest and disease resistance, abiotic stress tolerance, and herbicide resistance (Hajjar and Hodgkin 2007).

4.1.1.2 Pecan

Pecan (*Carya illinoinensis*), the most valuable native US nut crop (Grauke and Thompson 2008), was utilized and dispersed by Native Americans. In 2016, pecans were grown on 158,920 hectares in the USA and generated about 700 million dollars (USDA NASS 2017). Pecan was not domesticated until grafting techniques made crop improvement possible in the early 1900s (McWilliams 2013), and effective breeding efforts began in the latter part of the nineteenth century (Thompson and Grauke 1989). Today pecan is grown commercially on several continents (Grauke and Thompson 2008).

4.1.1.3 Blueberry

Among berry crops, blueberries are second only to strawberries in value of production ($748 million in 2016) in the USA (USDA NASS 2017). Two species native to the USA are the most commonly cultivated. Wild species of *Vaccinium* have played a significant role in the improvement of the cultivated blueberries in the USA from the beginning (Ballington 2009). Breeding of the northern highbush blueberry (*Vaccinium corymbosum* L.) began using plants selected from the wild in New Jersey in the early twentieth century, and most of the production is now from improved cultivars. Crosses initially involving *V. darrowii* Camp and *V. virgatum* Aiton with the northern highbush blueberry led to low chilling, heat- and drought-tolerant southern highbush cultivars adapted to the southeastern USA. The lowbush blueberry (*V. angustifolium* Aiton) is mainly harvested from managed native stands, although a few cultivars have been developed (Ballington 2009). In addition to the aforementioned species, breeders have also used *V. elliottii* Chapm. and *V. tenellum* Aiton in variety development (Ballington 2009).

4.1.1.4 Cranberry

The cultivated cranberry is derived from *Vaccinium macrocarpon* Aiton, the large-fruited cranberry, which occurs in bogs in eastern Canada and in 21 states in the northeastern and north-central USA and south to the Appalachian Mountains of eastern Tennessee and North Carolina (Stewart 1993). Virtually all commercially cultivated clones were originally selected from wild populations in only three states (Massachusetts, New Jersey, and Wisconsin). The small cranberry, *Vaccinium oxycoccos* L., is a secondary genetic relative of the cultivated cranberry, with a wide distribution in the northern hemisphere, including in the USA, from New England down to the Mid-Atlantic states, west to the Great Lakes states, and in the northwestern states as far south as the Cascade Mountains in Oregon.

4.1.1.5 Strawberry

The most commonly cultivated strawberry (*Fragaria ×ananassa*) is a hybrid of the wild Virginia strawberry (*F. virginiana* Mill.) and the beach strawberry (*F. chiloensis* (L.) Mill.). Both species are native to the USA, but the original cross took place in France in the eighteenth century and involved a traditional variety of the beach strawberry brought from Chile (Darrow 1966). Broad use was made of wild *F. chiloensis* and other wild species in the early twentieth century by Albert Etter, a private strawberry breeder in California (Clausen 1915). Etter recognized the value of the abundant variability in numerous characteristics, including vigor, productivity, flavor, and disease resistance in the wild species (Hancock and Luby 1993). In addition to the progenitor species of the cultivated strawberry, two other species of *Fragaria* are native to the USA: *F. cascadensis* K.E. Hummer, found in the Pacific Northwest, and *F. vesca* L., a cosmopolitan species.

4.1.1.6 Grapes

Grape (*Vitis vinifera* L.), the most valuable horticultural crop worldwide, is believed to have been domesticated in Anatolia over 6000 years ago (Cousins 2008). The muscadine grape (*Vitis rotundifolia* Michx.), a species native to the USA, is grown commercially on a much smaller scale than the wine grape and only in the southeastern USA. *Vitis labrusca* L., the fox grape, is native to the eastern USA and is the ancestor of many grape cultivars, including the Concord grape, which is used for juice and jam.

There are about 20 species of *Vitis* native to the USA (USDA, ARS 2017b), and many have been important genetic resources, especially as sources of insect and disease resistance. As described earlier in this chapter, the devastation of the European wine industry by phylloxera infestation in the 1800s was controlled through the use of mainly North American species to produce resistant rootstock (Gale 2002). *Vitis rotundifolia* is notable as a source of genes that provide resistances to several pests and diseases (Ferris et al. 2012). Grapevine powdery mildew (*Erysiphe necator* Schw. [synonym *Uncinula necator* (Schw.) Burr.]) resistance is found in *V. rupestris* Scheele, *V. aestivalis* Michx., *V. cinerea* (Engelm.) Millardet, *V. riparia,* and other native species (Alleweldt et al. 1990).

4.1.1.7 Wildrice

Three species of wildrice – *Zizania palustris* L., *Z. aquatica* L., and *Z. texana* – are native to the USA. *Zizania palustris* has a long history of being harvested and used as a staple food by Native Americans in the Great Lakes region. It was first cultivated in paddies in Minnesota in the 1950s (Oelke 1993), and breeding began in the 1960s, but domestication is still in its early phases (Oelke and Porter 2016). Most cultivation in the USA occurs in Minnesota and California. One variety of *Z. aquatica* occurs along streams from southern Ontario and Quebec south to the Atlantic and Gulf states, while another grows only along the St. Lawrence River estuary. *Zizania texana*, which is limited to a stretch of a few kilometers along one river in central Texas, is listed as endangered by the US Fish and Wildlife Service (USFWS; ECOS 2017). Both of these species, which are generally not utilized as food (Oelke 1993), are in the secondary genepool of *Z. palustris* (USDA, ARS 2017b).

4.1.1.8 Pawpaw

The pawpaw, *Asimina triloba* (L.) Dunal (Fig. 4.1), is the largest native fruit in the USA and occurs in 26 states in the eastern USA. It was likely dispersed from its original range by Native Americans (Small 2014). Breeding efforts to domesticate and improve the pawpaw occurred in the early 1900s, but it wasn't until the last decades of the century that serious progress was made (Cantaluppi 2015). Currently there is a resurgence of interest in pawpaw; however, challenges such as costly harvest, short shelf life, low yield, and development of processed products need to be addressed to make it commercially feasible on a large scale (Small 2014).

Fig. 4.1 Wild *Asimina triloba* (L.) Dunal (pawpaw) in West Virginia. (Photo: K.A. Williams)

4.1.1.9 Plum, Peach, and Cherry

Twenty-eight native species of *Prunus* occur in the USA, over half of which are plums (USDA, ARS 2017b). Plum species, including *P. americana* Marshall and *P. angustifolia* Marsh, have contributed to the improvement of the commercial Japanese plum (*P. salicina* Lindl.) (Okie 2000). *Prunus davidiana* (Carrière) N. E. Br., a native peach species, has contributed disease and pest resistance to the cultivated peach (Kervalla et al. 1998) and has been used to develop rootstocks (Okie 2000). Rapidly developing genomic techniques will expand the possibilities for use of the wild relatives to develop improved rootstock and scion cultivars (Potter 2011).

4.1.1.10 Raspberries and Blackberries

According to the GRIN-Global database, there are nearly 60 species of *Rubus* L. native to the USA (USDA, ARS 2017b). The American red raspberry (*Rubus idaeus* L. subsp. *strigosus* (Michx.) Focke) is included in the ancestry of most modern red raspberry cultivars. The black raspberry, *R. occidentalis* L., native to the eastern USA, is an important fruit crop in the USA, especially in Oregon, Ohio, Pennsylvania, and New York (Graham and Woodhead 2011). It has contributed numerous traits to improvement of the red raspberry, including heat tolerance, pest resistance, firm fruit, and late-ripening fruit (Finn and Hancock 2008). Purple raspberries, which are hybrids of red and black raspberries, are grown in some northeastern states. Blackberry species *R. alleghaniensis* Porter and *R. argutus* Link are among the ancestors of the most widely cultivated blackberries in the country (Small 2014).

4.1.1.11 Currants and Gooseberries

Genetic resources of the commercially important Eurasian black currant (*Ribes nigrum* L.), the European red currant (*R. rubrum* L.), and the European gooseberry (*R. uva-crispa* L.) abound in the USA. Forty-nine species of the nearly 180 species of *Ribes* L. occur in the country (USDA, ARS 2017b). Several of these species have contributed traits to improve production of the commercial species. Hybrids between the native North American northern gooseberry (*Ribes hirtellum* L.) and the European gooseberry make up most of the cultivated production of gooseberries in North America (Eizebroek and Wind 2008).

4.1.1.12 Walnut

Several of the six species of *Juglans* L. native to the USA have been used as rootstocks for the English walnut, *J. regia* L., which is cultivated for its nuts throughout the world. One of the species used as a rootstock either alone or as a hybrid with *J.*

regia is *J. hindsii* (Jeps.) R. E. Sm., the northern California black walnut, which is presumed by the California Native Plant Society to be extirpated or extinct in the wild (CNPS, Rare Plant Program 2017). *Juglans nigra* L., the American black walnut, produces the most valuable hardwood timber in the country, and its nuts are harvested from wild trees for use in candies, baked goods, and ice cream (McGranahan and Grant 2008). The wood of the winter-hardy butternut, *J. cinerea* L., is valuable and often used for wood carving and furniture (Eizebtoek and Wind 2008), but native stands are currently being decimated by a fungus that causes butternut canker (McGranahan and Grant 2008). The nuts were used extensively by Native Americans for food (Small 2014).

4.1.1.13 Medicinal Plants

More taxa of plants have been used as medicines than as foods by Native Americans (Moerman 1998). Some of these plants were adopted by European colonists (Heiser 1993), and many have contributed active ingredients to current pharmaceuticals (Prescott-Allen and Prescott-Allen (1986). Commercially important medicinal species that are native to the USA include American ginseng (*Panax quinquefolius* L.), goldenseal (*Hydrastis canadensis* L.), black cohosh (*Actaea racemosa* L.), and echinacea (*E. angustifolia* DC., *E. pallida* (Nutt.) Nutt., and *E. purpurea* (L.) Moench).

4.1.1.14 Forage and Turf Grasses

Many species of forage crops (forage grasses and forage legumes) and turf grasses are native to the USA. Notable genera include legumes (*Trifolium* L., *Lupinus* L., *Lotus* L., and *Astragalus* L.) and grasses (*Agrostis* L., *Bromus* L., *Festuca* L., and *Poa* L.) (Khoury et al. 2013).

4.1.1.15 Industrial Crops

The USA is home to the CWR of several genera of current or potential industrial crops. Among them are three species of cotton (*Gossypium* L.) and numerous species of meadowfoam (*Limnanthes* R. Br.). Well over 50 species of bladderpod or lesquerella (*Physaria* (Nutt. ex Torr. & A. Gray) A. Gray) also are native; the best known is *P. fendleri* (A. Gray) O'Kane & Al-Shehbaz, which is cultivated for an oil that can be used as a replacement for castor oil. The seven native species of guayule (*Parthenium* L.) include *P. argentatum* A. Gray in Texas, which is an alternate source of latex that is hypoallergenic and is of strategic importance to the US economy and military.

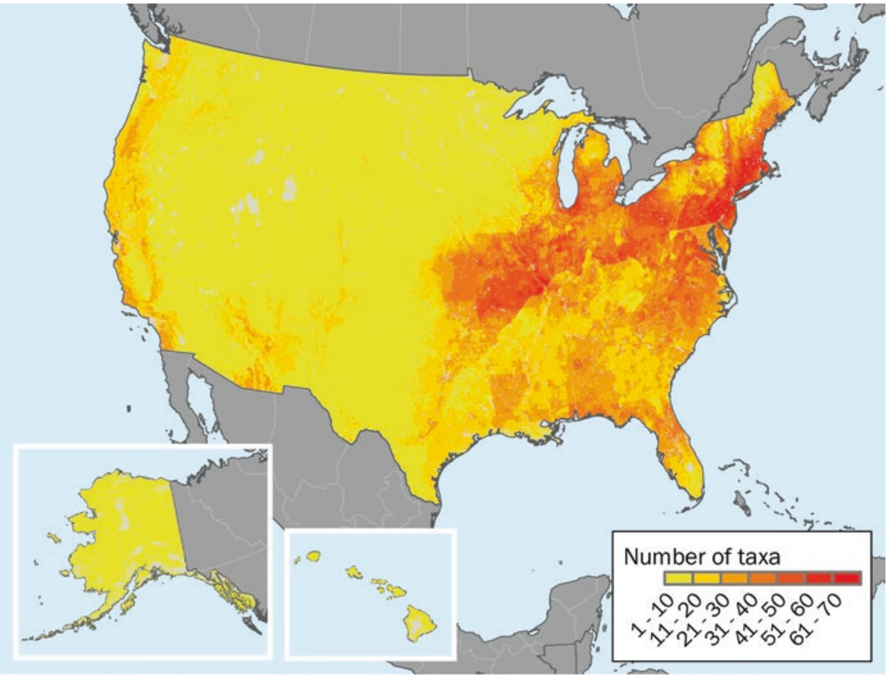


Fig. 4.2 Estimated potential richness map of 395 crop wild relatives in the USA. Warmer colors indicate where greater numbers of taxa potentially occur in the same geographic area. The full methods for generating the map and a list of reference data providers are given in Appendix 1

4.1.1.16 Species Richness of CWR in the USA

Figure 4.2 displays the richness of CWR in the USA based on modeled potential distributions of 395 CWR taxa covered in this book. According to this analysis, the greatest concentration of CWR occurs in the eastern and Midwestern USA.

4.2 US Plant Genetic Resource Conservation and Use Policies

The conservation of plant genetic resources (synonymous with plant germplasm) of CWR in the USA is shaped by agreements, strategies, and frameworks for biodiversity conservation and sustainable development at the international, national, and regional levels. The most influential of these are described in this section.

4.2.1 International Agreements

The US Government supports the implementation of several international agreements and guidelines that affect conservation and use of CWR, including the Second Global Plan of Action for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture (GPA) (FAO 2011), the Voluntary Guidelines to Support the Integration of Genetic Diversity into National Climate Change Adaptation Planning, and the United Nations 2030 Agenda for Sustainable Development (UN 2015). The history and status of ratification in the USA of the two international treaties that have had a substantial effect on the conservation and use of CWR are presented here. For more background on these treaties, refer to Chap. 1 in this book.

4.2.1.1 Convention on Biological Diversity (CBD)

The USA introduced the original resolution to the United Nations Environmental Program in 1989 that initiated negotiations to develop the CBD and was heavily involved in the drafting and negotiation phases. In spite of this, currently, the USA is one of only two member states of the United Nations that are not contracting parties to the CBD (CBD Secretariat 2017). The CBD was signed by US President William Clinton in June 1993 and sent to the US Senate for its advice and consent to ratification in November 1993, but the Senate did not act. Although not a contracting party to the CBD, the USA attends meetings of the Conference of the Parties. The State Department serves as the Access and Benefit Sharing National Focal Point and answers inquiries on obtaining access to genetic resources in the USA. Access to genetic resources is generally under the control of individual landowners. Because the USA is not a party to the CBD, the ratification of the Nagoya Protocol by the US government was not an option. However, US entities that obtain and utilize genetic resources from other countries for scientific or commercial purposes are subject to any requirements or restrictions instituted in these countries to implement the Nagoya Protocol.

4.2.1.2 International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA)

The USA actively participated in the development of the ITPGRFA, and President George W. Bush signed the Treaty in 2002. In 2008, the Executive Branch sought the advice and consent of the US Senate to ratification. The Senate Foreign Relations Committee heard testimony in support of ratification in 2009, but no action was taken then. In May 2016, the Committee again heard testimony and, in July 2016, forwarded the Treaty to the full Senate with a positive recommendation for advice and consent to ratification. After the US Senate gave its advice and consent, the

President ratified the Treaty on December 2, 2016, and the USA became the 143rd party on March 13, 2017. Genetic resources that are within the public domain and under the management and control of the US government, including those in the National Plant Germplasm System (NPGS), will be added to the Multilateral System established by the Treaty.

4.2.2 National and Regional Policies on Biodiversity Affecting Conservation and Use of Plant Genetic Resources

The current framework for biodiversity conservation in the USA is an assortment of strategies, agreements, laws, and regulations. The USA, as a nonparty to the CBD, does not have a comprehensive National Biodiversity Strategy or Action Plan, which have been developed by many countries as called for in Article 6 of the CBD. However, the USA does have a long history of protecting species and habitats.

Conservation and sustainable use of CWR, as components of biodiversity, are affected by general conservation actions in the USA. Among the frameworks and strategies developed for biodiversity conservation in the USA, the following are most relevant to the conservation of CWR.

4.2.2.1 National Framework for Progress

The Plant Conservation Alliance (PCA), a public-private consortium of 12 federal agencies and over 300 nonfederal cooperators, seeks to conserve native plants and their habitats and to ensure the sustainability of ecosystems. The 1995 PCA National Framework for Progress, intended to link resources and expertise in a coordinated national approach to plant conservation (https://s3.amazonaws.com/sitesusa/wp-content/uploads/sites/1193/2017/07/PCA-National-Framework-for-Progress.pdf), has six broad strategies and outlines goals and actions to implement the strategy at all levels. The Framework's strategy concerning conservation and restoration of native plants includes the conservation of CWR as an action item.

4.2.2.2 National Seed Strategy for Rehabilitation and Restoration 2015–2020

With the goal of helping to foster resilient and healthy landscapes, the National Seed Strategy (BLM 2015) was developed by the PCA Federal Committee, chaired by the US Department of the Interior Bureau of Land Management (BLM). It seeks to guide ecological restoration across major landscapes, especially for those lands damaged by rangeland fires, invasive species, severe storms, and drought, through an approach that coordinates the efforts of tribal, state, federal, local, and private entities. The Strategy emphasizes actions that increase the availability and informed use of genetically appropriate, locally adapted seeds to rehabilitate and restore native plant habitats. The importance of conserving native CWR and making them available for use is recognized in the Strategy.

4.2.2.3 North American Botanic Garden Strategy for Plant Conservation 2016–2020

The North American Botanic Garden Strategy for Plant Conservation 2016–2020 (BGCI 2016) is a regional approach by botanic gardens in the USA, Mexico, and Canada to implement the objectives of the Global Strategy for Plant Conservation (CBD 2012). A consortium of the American Public Gardens Association (APGA; which includes members in the USA, Canada, and other countries), the Association of Mexican Botanic Gardens, the US Office of Botanic Gardens Conservation International (BGCI), the Center for Plant Conservation (CPC), and the PCA supports the implementation of this Strategy. Conservation of plant genetic resources is specifically referenced in the vision statement as a component of the diversity of plant life the Strategy seeks to conserve. One of the targets in the strategy concerns conservation and preservation of economically and culturally important plants, including CWR, and cites the inventory of CWR of the USA (Khoury et al. 2013) as a resource.

4.2.3 Intellectual Property Rights

Intellectual property rights (IPR) protections that are claimed on the products of plant breeding in the USA fall into the main categories of plant variety protection or plant breeders' rights, plant patents, utility patents, trade secrets, and contracts (Kurtz et al. 2016). These protections cannot be applied directly to CWR, which, as naturally occurring unaltered plants, do not meet the criteria for IPR protection in the USA.

4.3 Ex Situ Conservation in the USA

4.3.1 National Germplasm Collections

The USDA-ARS has the primary responsibility in the USA for the ex situ preservation of germplasm collections of crop plants and their wild relatives from around the world, including those native to the USA. Other federal agencies, including the US

Forest Service (USFS), the Bureau of Land Management (BLM), and the Natural Resources Conservation Service (NRCS), have ex situ conservation roles related to a more limited set of CWR native to the USA.

4.3.1.1 The National Plant Germplasm System (NPGS)

The NPGS is responsible for the largest ex situ germplasm collection of crop plants and their wild relatives in the USA. A comprehensive review of the history of plant introduction and the early development of what became the US National Plant Germplasm System (NPGS) is provided by Janick (1989). The current structure of the NPGS emerged in 1974, two years after a reorganization of ARS (NRC 1991), as a network of Federal, State, and private organizations, coordinated by the US Department of Agriculture, Agricultural Research Service (ARS). State Agricultural Experiment Stations and 1862 Land-Grant Universities contribute substantial funds via "off-the-top" Hatch funds (federal agricultural multistate research funds), land, laboratory and office space, scientists, and support services. Private industry is an important source of funding for selected projects related to germplasm maintenance, regeneration, and evaluation.

As of September 2017, the NPGS collections included more than 580,000 accessions representing more than 15,600 species. In addition to the large number of accessions of globally important food and feed crops, the NPGS also maintains a wide variety of horticultural, fruit and nut, and industrial crops, as well as ornamental, medicinal, forest, and other plants (Bretting 2007). The collections include modern cultivars, inbred parental lines, elite breeding lines, landraces or farmer's varieties, wild relatives of crop species, wild species that may be needed for other purposes (revegetation, potential new crops, chemical analyses), and some rare and endangered species. Approximately 70% of the accessions are originally from foreign sources and 30% are of domestic origin. As of September 2017, approximately 80% of the accessions in the NPGS collections are available for distribution (USDA, ARS 2017a).

4.3.1.1.1 Structure of the NPGS

The NPGS collections are located at 19 sites around the country (Fig. 4.3). These sites acquire, maintain, regenerate, distribute, document, characterize, evaluate, and conduct research on the collections of specific plant genera under their management. The sites and collections are often classified as follows:

- Plant introduction (PI) station – One of the four original regional repositories responsible for multiple crop collections mainly of seed-reproducing species
- Clonal germplasm repository – A site responsible for the preservation of collections of perennial fruit, nut, grass, and ornamentals not easily seed-propagated, as well as of some seed-propagated woody landscape crops and wild species

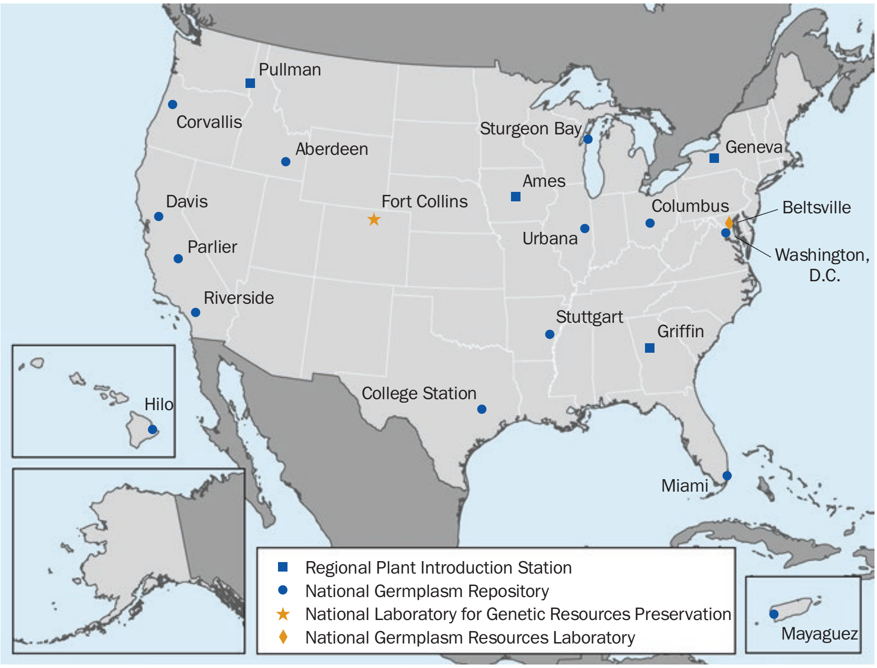


Fig. 4.3 The US National Plant Germplasm System

- Crop collection – A collection devoted to maintaining taxa related to a particular crop
- Genetic stock collection – Research collection of genetic mutants, cytological stocks with chromosomal aberrations, and sets of mapping populations and reference germplasm (some co-located with other types of collections)
- Specialized site – A site having a unique responsibility not included in the above categories

Accessions of native North American species are included in many of the collections. The collections and their locations, a listing of the primary crops or crop genepools (highlighting US CWR) maintained by each, and the number of accessions they currently hold (September 2017), are presented in Table 4.1.

4.3.1.1.2 Supporting Units of the NPGS

4.3.1.1.2.1 The National Laboratory for Genetic Resources Preservation (NLGRP)

The NLGRP in Ft. Collins, Colorado, maintains the primary security backup for NPGS germplasm in its “base collection.” As of September 2017, the NLGRP held in backup almost 430,000 accessions from the other NPGS sites. The Laboratory

Table 4.1 Sites and collections of the US National Plant Germplasm System[a]

Name and location	Type	Accessions[b]	Primary crop collections
North Central Regional Plant Introduction Station, Ames, Iowa	Plant introduction station	54,450	Maize, oilseed brassicas[c], cucurbits[c], sweet clover, amaranth[c], sunflower[c], millets[c], carrot[c], flax, woody ornamental species[c]
Plant Genetic Resources Unit, Geneva, New York	Plant introduction station, clonal germplasm repository	20,276	Tomato, vegetable brassicas, onion, grape[c], apple[c], sour cherry
Plant Genetic Resources Conservation Unit, Griffin, Georgia	Plant introduction station	100,042	Sorghum, sweet potato[c], peanut, cowpea, melons, pepper[c], mung bean, tropical and subtropical forage legumes[c], forage and turf grasses[c], subtropical and tropical squashes[c]
Western Regional Plant Introduction Station, Pullman, Washington	Plant introduction station	97,410	Common bean[c], onions[c], chickpea, temperate forage legumes[c], forage and turf grasses[c], lentil, lettuce, lupine[c], pea, safflower, sugar beet
National Small Grains Collection, Aberdeen, Idaho	Crop collection	140,229	Wheat, barley, oats, rice, rye, *Aegilops*, triticale
USDA Soybean Germplasm Collection, Urbana, Illinois	Crop collection	22,267	Soybean
National Cotton Germplasm Collection, College Station, Texas	Crop collection	9536	Cotton[c]
US Potato Genebank, Sturgeon Bay, Wisconsin	Crop collection	5963	Potato[c]
Ornamental Plant Germplasm Center, Columbus, Ohio	Crop collection	5111	Herbaceous ornamental species[c]
National Collection of Genetic Resources – Pecans and Hickories, Brownwood, Texas	Clonal germplasm repository	4066	Pecan[c], hickory[c], chestnut[c]
National Clonal Germplasm Repository, Corvallis, Oregon	Clonal germplasm repository	12,365	Pear, strawberry[c], raspberry[c], blackberry[c], blueberry[c], other small fruits[c], hop[c]
National Clonal Germplasm Repository – Tree Fruit and Nut Crops and Grapes, Davis, California	Clonal germplasm repository	8755	Grape[c], stone fruits[c], walnut[c], olive, pistachio[c], fig, persimmon[c], mulberry[c], kiwi, pomegranate

(continued)

Table 4.1 (continued)

Name and location	Type	Accessions[b]	Primary crop collections
Tropical Plant Genetic Resources and Disease Research Unit, Hilo, Hawaii	Clonal germplasm repository	850	Macadamia, pineapple, papaya, lychee, jackfruit, breadfruit, carambola, guava, passion fruit, rambutan
Clonal Repository for Tropical and Subtropical Germplasm, Puerto Rico	Clonal germplasm repository	1159	Banana, cacao, tropical fruits[c]
Subtropical Horticulture Research Station, Miami, Florida	Clonal germplasm repository	3255	Sugarcane, mango, avocado[c], ornamentals
National Clonal Germplasm Repository for Citrus and Dates, Riverside, California	Clonal germplasm repository	1800	Citrus and related genera, dates
Woody Landscape Plant Germplasm Repository, Washington, D.C.	Clonal germplasm repository	7947	Woody ornamental species[c]
Barley Genetic Stock Collection, Aberdeen, Idaho	Genetic stock collection	3349	Barley
Maize Genetics Cooperation Stock Center, Urbana, Illinois	Genetic stock collection	8127	Maize
G.A. Marx Pea Genetic Stock Collection, Pullman, Washington	Genetic stock collection	712	Pea
Genetic Stocks *Oryza* Collection, Stuttgart, Arkansas	Genetic stock collection	37,541	Rice
C.M. Rick Tomato Genetics Resource Center, Davis, California	Genetic stock collection	3731	Tomato
Wheat Genetic Stock Collection, Aberdeen, Idaho	Genetic stock collection	401	Wheat
National Arid Land Plant Genetic Resources Unit, Parlier, California	Specialized (warm-season arid environment)	1496	Bladderpod[c], meadowfoam[c], prickly pear[c], guayule[c], jojoba[c]

[a] Some at the same location

[b] As of September 2017

[c] Crop collections with significant accessions of US CWR

also manages the safety backup storage for designated non-NPGS plant germplasm. Since 2008, the Laboratory has coordinated shipments of NPGS accessions to the Svalbard Global Seed Vault, administered by Norway's Nordic Genetic Resources Center and the Global Crop Diversity Trust. Research is conducted at the Laboratory to develop strategies and technologies to increase the efficiency and effectiveness of plant genebanks. This includes research on the best methods for secure long-term preservation of plant germplasm, including cryopreservation, and sampling and conserving the genetic diversity of plant populations, as well as genes and specific genotypes, in diverse germplasm forms.

4.3.1.1.2.2 The National Germplasm Resources Laboratory (NGRL)

The NGRL in Beltsville, Maryland, provides services in support of the entire NPGS, particularly in the activities of germplasm acquisition, introduction, documentation, and distribution. The NGRL assists in the identification, prioritization, and acquisition of germplasm, facilitates international germplasm exchange, manages a plant exploration and exchange program to acquire new germplasm, and coordinates with the Animal and Plant Health Inspection Service (APHIS) on importing and exporting germplasm. The Laboratory also develops and operates GRIN-Global and provides physical and electronic security for the hardware on which the GRIN-Global database and software reside. In addition, the NGRL acts as the coordinator and secretariat for the 42 NPGS Crop Germplasm Committees (CGCs) described later in this section.

4.3.1.1.3 Documentation of NPGS Collections and CWR

4.3.1.1.3.1 GRIN-Global

Since the early 1980s, passport, taxonomic, evaluation, inventory, and distribution data on NPGS collections have been maintained in GRIN, a centralized database readily accessible via the Internet. In 2008, development of a new scalable and flexible version of GRIN called GRIN-Global was initiated through the cooperation of the Global Crop Diversity Trust, Bioversity International, and USDA-ARS. The first version of GRIN-Global was released in 2011 and made available for use by any genebank in the world. GRIN-Global provides a complete genebank information management system with extensive opportunities for customization. In 2015, the NPGS version (https://www.ars-grin.gov/npgs/index.html) of GRIN-Global was put into production. Relative to the CWR of the USA, the NPGS GRIN-Global provides information on current germplasm holdings and evaluation data, taxonomy, geographic distribution, designation of conservation concern either at the federal or state level, noxious weed designation, and links to multiple additional websites with information.

4.3.1.1.3.2 GRIN Crop Wild Relative Project

Also included in GRIN-Global are the results of an ongoing project focused on the CWR of the world and their importance as genetic resources (Wiersema and León 2016). This project expands on the standard information on species already included in GRIN-Global to classify their potential to donate genes for crop improvement. The starting point for the method of classification of CWR is the genepool concept of Harlan and de Wet (1971), which is useful when the hybridization potential of a CWR with a crop has been studied. When crossability data do not exist, the degree of genetic relatedness is estimated through other means, incorporating the "taxon group" concept developed by Maxted et al. (2006), which is based on taxonomic relationship of the CWR with the crop, and additional information, such as phylogenetic data, ploidy level, reproductive biology, and natural hybridization/introgression (Wiersema and León 2016). The classes of relatedness of CWR to the crop are designated as primary, secondary, and tertiary "genetic relative" classes rather than as "genepools" because they are not based solely on crossing ability. Also, a fourth class of genetic relatives that are important for graft stock (especially rootstock) breeding was added.

Over eighty crops with native or naturalized CWR in the USA have been treated in the GRIN CWR Project, including amaranth, apple, beans, blueberries, carrot, cotton, onion, pecan, potato, strawberry, clovers, cranberries, grapes, hop, lettuce, maize, millets, peach, pistachio, plum, quinoa, pumpkins and squashes, raspberries, sunflower, sweet cherry, sweet potato, walnuts, wildrice, and yam (Wiersema and León 2016). These CWR treatments are useful for a number of applications, including identifying gaps in germplasm collections and setting priorities for conservation actions, both ex situ and in situ. The data are available through the GRIN-Global Taxonomy website (https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomyquery.aspx).

4.3.1.1.4 Distribution of Germplasm by the NPGS

Over 250,000 accessions of germplasm, the largest number of any national genebank, are distributed yearly to recipients around the world. Distributions of accessions of native CWR in the NPGS are made according to the same policies in place for other accessions, namely, that all qualified users worldwide are provided unrestricted access to germplasm that is within the public domain and under the management and control of the USDA-ARS for use in research, breeding, and education. Future international distribution of these accessions will be governed by the terms of the Standard Material Transfer Agreement (SMTA) now that the USA is a contracting party to the ITPGRFA and the collections are included in the Treaty's Multilateral System.

4.3.1.1.5 Crop Germplasm Committees (CGCs)

A key component of the NPGS is the group of 42 CGCs (https://www.ars-grin.gov/npgs/cgclist.html) that provide technical input to the managers, curators, and staffs of the NPGS sites on a specific crop or group of related crops. These committees are composed of customers and stakeholders from government, academic, and private organizations and include specialists from a range of pertinent scientific disciplines. The CGCs' functions include analysis of the composition of the NPGS collections, making recommendations and participating in the acquisition of additional germplasm, advising curators on cultural and regeneration procedures, and developing descriptors for germplasm characterization and evaluation. CGC members collaborate with curators to develop crop vulnerability statements that provide critical information for setting priorities for NPGS activities. The CGCs review proposals and endorse plant explorations sponsored by the NPGS Plant Exploration Program for genetic resources within the purview of their committees.

4.3.1.2 Other Federal and University Collections Affiliated with the NPGS

Three other germplasm collections are closely associated with the NPGS.

4.3.1.2.1 Desert Legume Program (DELEP)

The DELEP (http://cals.arizona.edu/desertlegumeprogram/), a joint project of the University of Arizona College of Agriculture and Life Sciences and the Boyce Thompson Arboretum in Tucson, Arizona, focuses on collection, preservation, and study of desert legume species from the SW USA and around the world. The collection includes almost 4000 accessions of 1425 species from 66 countries (personal communication, Matthew Johnson, 12 August 2016), with backup provided by the NPGS at the NLGRP. An Index Seminum lists the samples that are available for distribution around the world. A snapshot of accessions in the DELEP collections is available through GRIN-Global, but as of this writing, data on the accessions, including availability, is not fully integrated. Crop wild relatives in the DELEP collection that are native or naturalized in the USA belong to the genera *Lathyrus* L. (3 species), *Lupinus* (24 species), *Phaseolus* L. (6 species), *Trifolium* (2 species), and *Vicia* L. (2 species).

4.3.1.2.2 US *Nicotiana* Germplasm Collection

The US *Nicotiana* Germplasm Collection is maintained by North Carolina State University in Raleigh. Lewis and Nicholson (2007) have documented the history of the development of the collection. The collection includes over 2200 accessions of

65 taxa in 62 species of *Nicotiana* L. Although the collection is not a part of the NPGS, *Nicotiana* germplasm is backed up at the NLGRP, and germplasm can be ordered through GRIN-Global, which has complete information on the accessions. The collection includes domestic cultivars, wild species, and interspecific hybrids. The wild species collection includes 62 accessions of 5 species native to the USA.

4.3.1.2.3 National Seed Laboratory (NSL)

The USFS NSL, currently located in Dry Branch, Georgia, conserves seed of native plants in the USA for ecosystem conservation and restoration (Karrfalt 2006). Seed from the NSL is backed up at the NLGRP. All samples are documented in GRIN-Global, but seeds are ordered by directly contacting the NSL. The collection consists of 7600 accessions of 107 taxa (103 species), ranging from commercial timber species to herbaceous understory species. Species of trees in the genera *Chamaecyparis* Spach, *Cupressus* L., *Fraxinus* L., *Juniperus* L., *Pinus* L., *Tsuga* (Endl.) Carrière, and *Robinia* L., as well as a few nontimber native species, are included. Most accessions originated in the USA, with a few from Canada.

4.3.1.3 NPGS Acquisition and Holdings of CWR and WUS

4.3.1.3.1 NPGS Plant Explorations for CWR in the USA

USDA plant exploration began in earnest in 1898 with the establishment of the Section of Seed and Plant Introduction that initially focused exclusively on collection of plants in other countries. It was not until half a century later that USDA-supported explorations began in the USA. The Research and Marketing Act (RMA) of 1946, which resulted in the establishment of the four regional PI stations, directed that research on utilization include native as well as foreign plants and authorized funds for this purpose. Thus, beginning in 1953, the ARS administrative regions served by the PI stations mounted plant explorations in the USA with funds from the USDA New Crops Research Branch, which managed USDA plant explorations at the time. The first targets were native grasses, legumes, and shrubs (Burgess 1971).

Today USDA plant explorations are supported and coordinated by the NGRL in Beltsville, Maryland. Support for plant explorations is contingent upon endorsement by the CGC that represents experts on the crop genepool. Based on the records of the NGRL, between 1953 and 2017, most of the 217 explorations that took place in the USA targeted native wild species (Table 4.2 and Fig. 4.4). In the past two decades, both the number and the percentage of NPGS explorations that are domestic have increased as the obstacles to conducting explorations in some other countries have compounded (Williams 2005) and the recognition of the importance of native CWR in the US has increased. The numbers of NPGS domestic explorations vary widely for each CGC. Explorations supported by funding sources other than the NGRL are not included in the table.

Table 4.2 NPGS plant explorations (1953–2017) for CWR in the USA grouped by Crop Germplasm Committee [ab]

CGC	Major native or naturalized US genera under CGC responsibility	Number of explorations for CWR in the USA 1953–2017	Major CWR taxa collected on NPGS explorations in the USA	Notes
Apple	*Malus* Mill.	5	*Malus angustifolia* (Aiton) Michx., *M. coronaria* (L.) Mill., *M. fusca* (Raf.) C. K. Schneid.	
Barley	*Hordeum* L.	0		
Carya	*Carya* Nutt.	2	*Carya glabra* (Mill.) Sweet, *C. floridana* Sarg., *C.* spp.	
Clover and special purpose legumes	*Aeschynomene* L., *Astragalus* L., *Desmanthus* Willd., *Desmodium* Desv., *Glycyrrhiza* L., *Lathyrus* L., *Lespedeza* Michx., *Lotus* L., *Lupinus* L., *Trifolium* L., *Vicia* L.	5	*Astragalus* L., *Trifolium* L., *Lathyrus* L., *Lotus* L., *Lupinus* L., *Oxytropis* DC. spp.	
Cotton	*Gossypium* L.	0		
Crucifer	*Brassica* L.	0		
Cucurbits	*Cucurbita* L.	3	*Cucurbita okeechobeensis* (Small) L. H. Bailey subsp. *okeechobeensis, C. digitata* A. Gray, and *C. palmata* S. Watson	
Forage and turf grass	*Achnatherum* P. Beauv., *Andropogon* L., *Bouteloua* Lag., *Bromus* L., *Elymus* L., *Festuca* L., *Panicum* L., *Pascopyrum* Á. Löve, *Paspalum* L., *Poa* L., *Pseudoroegneria* (Nevski) Á. Löve, *Schizachyrium* Nees, *Sorghastrum* Nash, and others	20	Many species	

Grape	*Vitis* L.	4	*Vitis aestivalis* Michx., *V. cinerea* (Engelm.) Millardet, *V. labrusca* L., *V. monticola* Buckley, *V. mustangensis* Buckley, *V. palmata* Vahl, *V. riparia* Michx., *V. rotundifolia* Michx., *V. shuttleworthii* House, *V. vulpina* L.	
Herbaceous ornamental	*Asclepias* L., *Begonia* L., *Coreopsis* L., *Lilium* L., *Phlox* L., *Rudbeckia* L., *Viola* L., and others	10	*Arctostaphylos* Adans., *Begonia* L., *Ceanothus* L., *Coreopsis* L., *Lilium* L., *Monardella* Benth., *Phlox* L., *Rudbeckia* L., *Salvia* L., *Stokesia* L'Hér. spp.	
Juglans	*Juglans* L.	3	*Juglans cinerea* L., *J. major* (Torr.) A. Heller, *J. microcarpa* Berland., *J. nigra* L.	
Leafy vegetable	*Lactuca* L., *Micromonolepsis* Ulbr., *Blitum* L.	3	*Blitum* L., *Micromonolepsis* Ulbr., and *Suckleya* A. Gray spp.	
Maize	*Tripsacum* L.	0		
Medicinal	*Actaea* L., *Agave* L., *Echinacea* Moench, *Hesperaloe* Engelm., *Hydrastis* J. Ellis, *Mentha* L., *Oenothera* L., *Panax* L., *Prunella* L., *Salvia* L., *Sambucus* L., *Scutellaria* L., *Serenoa* Hook. f., *Tanacetum* L., *Ulmus* L., *Yucca* L.	29	Many species	Many conducted in cooperation with the National Cancer Institute natural products drug discovery program (about 1960–82)

(continued)

Table 4.2 (continued)

CGC	Major native or naturalized US genera under CGC responsibility	Number of explorations for CWR in the USA 1953–2017	Major CWR taxa collected on NPGS explorations in the USA	Notes
New crops	*Agave* L., *Amaranthus* L., *Asclepias* L., *Chenopodium* L., *Crotalaria* L., *Cucurbita* L., *Cuphea* P. Browne, *Amaranthus* L., *Euphorbia* L., *Grindelia* Willd., *Hibiscus* L., *Limnanthes* R. Br., *Opuntia* Mill., *Parthenium* L., *Physaria* (Nutt. ex Torr. & A. Gray) A. Gray, *Prosopis* L., *Salicornia* L., *Salvia* L., *Simmondsia* Nutt., *Stokesia* L'Hér., *Taraxacum* F. H. Wigg.	22	Many species	
Peppers	*Capsicum* L.	0		
Phaseolus	*Phaseolus* L.	5	*Phaseolus acutifolius* A. Gray, *P. polystachios* (L.) Britton et al.	
Potato	*Solanum* L.	16	*Solanum jamesii* Torr., *S. stoloniferum* Schltdl.	
Prunus	*Prunus* L.	4	*Prunus andersonii* A. Gray, *P. fasciculata* (Torr.) A. Gray, *P. ilicifolia* (Nutt. ex Hook. & Arn.) D. Dietr., *P. subcordata* Benth., *P. umbellata* Elliott	
Root and bulb	*Allium* L., *Daucus* L.	1	*Daucus* L. spp.	
Small fruits	*Fragaria* L., *Ribes* L., *Rubus* L., *Vaccinium* L.	23	*Fragaria* L., *Ribes* L., *Rubus* L., *Sambucus* L., *Vaccinium* L. spp.	
Sorghum	*Sorghum* Moench	1	*Sorghum halepense* (L.) Pers.	Exploration for naturalized *Sorghum* species

Specialty nuts	*Castanea* Mill, *Corylus* L., *Pistacia* L.	1	*Corylus americana* Marshall, *C. californica* (A. DC.) Rose	
Sugar beet	*Beta* L. (naturalized), *Aphanisma* Nutt. ex Moq.	1	*Beta vulgaris* L. subsp. *maritima* (L.) Arcang., *B. macrocarpa* Guss.	Exploration for naturalized *Beta* spp.
Sunflower	*Helianthus* L.	22	Many *Helianthus* L. spp.	
Sweet potato	*Ipomoea* L.	3	*Ipomoea* L. spp.	
Tobacco	*Nicotiana* L.	0		
Tropical fruit and nut	*Carica* L., *Persea* Mill., *Psidium* L.	0		
Woody landscape plant	Many	34	*Betula* L. spp., *Cornus* L. spp., *Cotinus obovatus* Raf., *Diervilla lonicera* Mill., *Fraxinus* L. spp., *Gymnocladus dioicus* (L.) K. Koch, *Magnolia ashei* Weath., *Quercus muehlenbergii* Engelm., *Spiraea alba* Du Roi, *Tabebuia haemantha* (Bertero ex Spreng.) DC., and others	

[a] Includes only those CGCs with a mandate for crops with major CWR native or naturalized in the USA

[b] One exploration was conducted for *Humulus lupulus* var. *neomexicanus*, a CWR of hop, which does not have a CGC

Fig. 4.4 *Phaseolus polystachios* (L.) Britton et al. subsp. *polystachios* collected in Brevard County, Florida on an NPGS plant exploration in 2010. (Photo: K.A. Williams)

4.3.1.3.2 Holdings of Native CWR and WUS

At this writing, the NPGS maintains more than 36,000 accessions of wild plants collected in the USA (USDA, ARS 2017a). Of these, 4501 accessions (documented as collected in the wild in the USA) represent 164 taxa that were identified as high priority (Priority 1A) CWR and WUS taxa in the national inventory (Table 4.3).

4.3.1.4 Other Federal Germplasm Collections

In addition to the USDA-ARS, other agencies in the US government are involved in the collection and conservation of native plants, many of which are CWR.

4.3.1.4.1 Seeds of Success (SOS)

The SOS program, led by the BLM, supports the collection of seeds in the USA from wild genetically diverse populations of native plants important for restoration (Haidet and Olwell 2015). The main focus of SOS is on species most threatened by climate change and species representing key ecological communities. The BLM has many collecting partners, including botanic gardens and other federal and nonfederal institutions. About 50 teams based at BLM, USFWS offices, and botanic gardens across the USA make collections following a standard protocol (Haidet and Olwell 2015). The program is an integral part of BLM's Native Plant Materials Development Program, which has the objective of making genetically appropriate native seeds and plants more available for restoration work.

Table 4.3 NPGS accessions of taxa with Priority 1A in the national inventory of CWR in the USA

Related crop (s)/WUS	Related crop(s)/WUS scientific name	Botanical family	Number of P1A CWR taxa native to the USA[a]	Number of P1A CWR taxa represented in NPGS[b]	Total number of accessions of P1A CWR collected in the USA[b]
Bean (tepary)	*Phaseolus acutifolius* A. Gray var. *acutifolius*	Fabaceae	2	2	167
Blueberry/cranberry/ lingonberry	*Vaccinium angustifolium* Aiton, *V. corymbosum* L., *V. virgatum* Aiton/*V. macrocarpon* Aiton/*V. vitis-idaea* L.	Ericaceae	22	20[c]	559
Chinese or American Chestnut	*Castanea mollissima* Blume, *C. dentata* (Marshall) Borkh.	Fagaceae	2	2	3[d]
Currants/gooseberries	*Ribes nigrum* L., *R. rubrum* L./*R. uva-crispa* L.	Grossulariaceae	23	21	145
Fig	*Ficus carica* L.	Moraceae	1	1	1
Guava	*Psidium guajava* L.	Myrtaceae	1	1	3
Hazelnut	*Corylus avellana* L.	Betulaceae	3	3	69
Allium crops (leek/ garlic/\Welsh onion/ ramp)	*Allium porrum* L. *Allium sativum* L. *Allium fistulosum* L. *Allium tricoccum* Aiton	Amaryllidaceae	3	2	3
Lettuce	*Lactuca sativa* L.	Asteraceae	10	7	24
Maize	*Zea mays* L. subsp. *mays*	Poaceae	3	1	77
Mate	*Ilex paraguariensis* A. St.-Hil.	Aquifoliaceae	3[e]	3	12
Pecan	*Carya illinoinensis* (Wangenh.) K. Koch	Juglandaceae	9	9	127[f]

(continued)

Table 4.3 (continued)

Related crop (s)/WUS	Related crop(s)/WUS scientific name	Botanical family	Number of P1A CWR taxa native to the USA[a]	Number of P1A CWR taxa represented in NPGS[b]	Total number of accessions of P1A CWR collected in the USA[b]
Peppers	*Capsicum annuum* L. *C. chinense* Jacq. *C. frutescens* L. *C. baccatum* L. var. *pendulum* (Willd.) Esbaugh *C. pubescens* Ruiz & Pav.	Solanaceae	1	1	1
Persimmon	*Diospyros kaki* Thunb.	Ebenaceae	2	2	8
Pumpkins and squashes (pepo/ovifera/cushaw/butternut)	*Cucurbita pepo* subsp. *pepo* L./*Cucurbita pepo* subsp. *ovifera*/*C. argyrosperma* C. Huber subsp. *argyrosperma*/*C. moschata* Duchesne	Cucurbitaceae	4	4	77
Pistachio	*Pistacia vera* L.	Anacardiaceae	1	1	10
Potato	*Solanum tuberosum* L.	Solanaceae	1	1	248
Prunus (cherry/peach/plum)	*Prunus avium* (L.) L., *P. cerasus* L./*P. persica* (L.) Batsch/*P. salicina* Lindl., *P. domestica* L. subsp. *domestica*	Rosaceae	16	5	22
Raspberries/blackberries	*Rubus idaeus L subsp. idaeus.*, *R. occidentalis* L./*R. allegheniensis* Porter, *R. argutus* Link, *R. pensilvanicus* Poir., *R. ursinus* Cham.& Schtdl.	Rosaceae	40	17	378
Star-anise	*Illicium verum* Hook. f.	Schisandraceae	1	1	3
Strawberry	*Fragaria ×ananassa* Duchesne ex Rozier	Rosaceae	8	8	518
Sugar maple	*Acer saccharum* Marshall	Sapindaceae	2	2	6
Sunflower	*Helianthus annuus* L.	Asteraceae	19	19	1516
Sweet potato	*Ipomoea batatas* (L.) Lam. var. *batatas*	Convolvulaceae	8	4[g]	4
Upland cotton	*Gossypium hirsutum* L.	Malvaceae	3	3	59

Vanilla	*Vanilla planifolia* Andrews	Orchidaceae	2	0	0
Walnut	*Juglans nigra* L.	Juglandaceae	5	5	128
Wildrice	*Zizania palustris* L.	Poaceae	4	0	0
Wine grape	*Vitis vinifera* L.	Vitaceae	26	19	333

[a] Source: Khoury et al. 2013

[b] Source: USDA, ARS 2017a

[c] Including *V. ovalifolium*, for which the accessions are not identified to the variety level

[d] Actual number of accessions of chestnut CWR is higher, but data are incomplete in GRIN-Global

[e] Accessions identified only to species level

[f] Actual number of accessions of pecan CWR is higher, but data are incomplete in GRIN-Global

[g] All accessions are not identified to the variety level in GRIN-Global

Seed samples collected by BLM teams are sent to the NPGS site in Pullman, Washington, for incorporation into the NPGS and documentation in the GRIN-Global database. Samples are made available to crop-specific curators in the NPGS for incorporation into their collections and are also sent to the NLGRP for long-term security backup. Since 2001, more than 16,000 collections of over 5000 taxa have been made under the SOS program. A number of the taxa collected by the program are CWR. A comparison of the priority CWR taxa from Khoury et al. (2013) to the SOS Collection revealed that, as of January 2017, 92 samples representing 39 taxa of Priority 1A (native, closely related to major crops) in 14 genera have been collected. The species with the greatest number of collections are *Helianthus annuus* (21 samples), followed by *Helianthus petiolaris* (10 samples), *Prunus emarginata* (Douglas) Eaton (9 samples), and *Lactuca floridana* (L.) Gaertn. (4 samples).

4.3.1.4.2 Natural Resources Conservation Service Plant Materials Centers (PMCs)

The NRCS operates 25 PMCs, which collect, evaluate, select, and release native and introduced plant material to address soil and water conservation problems and improve pasture and rangelands. Most of the new releases are native species (John Englert, personal communication, 10 August 2017). In a review of current releases (USDA NRCS 2017a), 33% were found to be Priority 1 or 2 CWR species as defined in the national inventory of US CWR (Khoury et al. 2013). Most were grasses, such as big blue stem (*Andropogon gerardi* Vitman), sand blue stem (*A. hallii* Hack.), wild rye and wheat grass species [*Elymus canadensis* L., *E. elymoides* (Raf.) Swezey, *E. glaucus* Buckley, *E. lanceolatus* Scribn. & J.G. Sm.) Gould, and *E. virginicus* L.], switch grass (*Panicum virgatum* L.), and eastern gamagrass [*Tripsacum dactyloides* (L.) L.]. Selections from native populations of cherries and plums (*Prunus americana*, *P. angustifolia*, *P. maritima* Marshall, *P. pumila* L., and *P. virginiana* L.) have also been released for wildlife habitat and erosion control. The NRCS releases are made available to commercial producers and are discontinued when replaced by more effective material or when there is minimal commercial interest (USDA NRCS 2017b). Agency policy requires that all NRCS releases are deposited in the NPGS (John Englert, personal communication, 10 August 2017). The PMCs are also encouraged to offer initial collections (non-released material) to NPGS curators if adequate seed is available. The National Plant Materials Center in Beltsville, Maryland, coordinates contacts with the NPGS curator or the NLGRP for deposition of samples.

4.3.1.4.3 USFWS Conservation of Wildrice

Texas wildrice (*Zizania texana*), a secondary genetic relative of wildrice endemic to the upper reaches of the San Marcos River in Texas, is listed as endangered by the USFWS (ECOS 2017). As a backup for this rare species, the USFWS maintains living plants representing the allelic richness and diversity of the *Z. texana* populations at the San Marcos Aquatic Resources Center, San Marcos, Texas, and the Southwestern Native Aquatic Resources and Recovery Center in Dexter, NM (Richards et al. 2007; Wilson et al. 2017). Methods for ex situ conservation of *Z. texana* and other *Zizania* species are available (Porter 2018; Chap. 14, this book) but are labor intensive (Walters et al. 2018; Chap. 10, this book) and have not been standard practice.

4.3.2 State Germplasm Collections

Germplasm collections including native CWR are located at some universities, and other facilities are supported by state governments. Some of the best known collections are described here.

4.3.2.1 The Pawpaw Collection at Kentucky State University

The pawpaw collection at Kentucky State University serves as a satellite site of the USDA-ARS Clonal Repository at Corvallis, Oregon. The collection of pawpaws includes two species, *Asimina triloba* Kral and *A. longifolia* (L.) Dunal, and two hybrids, *A. triloba* × *A. obovata* and *A. triloba* × *A. reticulata* (Sheri Crabtree, personal communication, January 23, 2017). Included are approximately 1000 *A. triloba* accessions, with about 850 accessions from throughout the native range, 25 cultivars, and 125 crosses and advanced selections. Accessions of *A. triloba* are available upon request. Cultivars are the most frequently requested accessions.

4.3.2.2 The Greenbelt Native Plant Center

The Greenbelt Native Plant Center (http://www.marsb.org/) provides seeds of plants collected from local populations for conservation and restoration projects in New York City and the surrounding area. The Mid-Atlantic Regional Seed Bank (MARS-B), a unit of the Greenbelt Native Plant Center, is a partner in the SOS program; a portion of every seed sample collected is deposited with the NPGS. Although their focus is on collecting species needed for restoration projects in the eastern USA, some of the targets include CWR, such as those in the genera *Helianthus* L., *Ipomoea* L., *Fragaria* L., and *Prunus* L.

4.3.2.3 Center for Agroforestry at the University of Missouri

The Center for Agroforestry (http://www.centerforagroforestry.org/mission.php) at the University of Missouri maintains germplasm collections of eastern black walnut and northern pecan to support tree improvement programs. At this writing, the exact numbers of accessions of wild material were not available. Scientists at the Center conduct research to develop genetically improved cultivars of these and other crops as new specialty crops for growers in Missouri and the Midwest.

4.3.3 Nongovernmental Organizations

In recent years, botanic gardens and arboreta have embraced an expanded role in the conservation of CWR (Miller et al. 2015). According to BGCI, there are over 800 botanic gardens and arboreta in the USA (https://www.bgci.org/garden_search.php). Crop wild relatives are conserved in the collections of many of these organizations. These institutions also play a role in educating the public on the importance of CWR with displays and programs that inform visitors about the links between wild plants and agricultural crops. In addition, preserved plant specimens in the herbaria of gardens and other institutions are a critical source of information on the geographic distribution and variation in CWR species. Some of the nongovernmental institutions and networks most active in the conservation of CWR are described below. In most cases, CWR that are conserved are included because of their threat status rather than their role as CWR.

4.3.3.1 Center for Plant Conservation (CPC)

The CPC, a nonprofit organization based at the San Diego Zoo Global, coordinates a network of over 40 botanical gardens and arboreta across the USA that are conserving and restoring the rare and endangered native plants of the USA (CPC 2017). The CPC's National Collection of Endangered Plants contains samples of more than 780 of the country's most imperiled plants. Plants are added to the National Collection by participating institutions, which collect live material from wild populations in their regions of the country and maintain it as seeds, rooted cuttings, or mature plants. Material from the National Collection is provided to governmental agencies and private land-management organizations to establish new populations and for use in research and education. The USDA-ARS NLGRP provides backup storage of seeds of plants in the National Collection, but these are not part of the NPGS. The CWR taxa included in the National Collection that were ranked as priorities for conservation in the USA in Khoury et al. (2013) are listed in Table 4.4. For some of these taxa that are not currently conserved in the NPGS (see last column), the CPC institutions may have the only ex situ collections.

4.3.3.2 Native Seeds/SEARCH (NS/S)

Located in Tucson, Arizona, NS/S (http://www.nativeseeds.org/our-approach/seed-bank) is a nonprofit organization that conserves and makes available crop genetic resources from the SW USA and northwestern Mexico through both ex situ and in

Table 4.4 CPC collection (CPC 2017) representatives of Priority 1A and 1B CWR taxa in the national inventory

Taxon/priority ranking in US CWR national inventory[a]	Crop	No. of CPC participating institutions	USFWS status[b]	Accessions of taxon in the NPGS[c]
Allium munzii (Ownbey & Aase ex Traub) McNeal/P1B	Onion	1	E	0
Cucurbita okeechobeensis (Small) L. H. Bailey subsp. *okeechobeensis*/P1A	Pumpkin, squash	1	E	9
Helianthus carnosus Small/P1B	Sunflower	1	SC	5
Helianthus eggertii Small/P1B	Sunflower	1	D	12
Helianthus niveus (Benth.) Brandegee subsp. *tephrodes* (A. Gray) Heiser/P1A	Sunflower	1	D	11
Helianthus paradoxus Heiser/P1A	Sunflower	3	T	13
Helianthus schweinitzii Torr. & A. Gray/P1B	Sunflower	1	E	2
Ilex collina Alexander/P1B	Yerba mate	1	RT	0
Manihot walkerae Croizat/P1B	Cassava	1	E	0
Prunus geniculata R. M. Harper/P1B	Plum, Japanese plum, myrobalan plum, apricot	1	E	1
Ribes echinellum (Coville) Rehder/P1B	Currant, gooseberry	2	T	3
Solanum sandwicense Hook. & Arn./P1B	Potato, eggplant, tomato	1	E	0
Vicia menziesii Spreng./P1B	Faba bean, vetch	1	E	0
Zizania texana Hitchc./P1A	Wildrice	2	E	0

E endangered, *T* threatened, *D* delisted taxon, *RT* resolved taxon, *SC* species of concern

[a] Priorities from Khoury et al. (2013)

[b] USFWS Status Code Definitions (from https://www.fws.gov/endangered/about/listing-status-codes.html)

[c] Accessions publicly available in the NPGS (not related to the backup of CPC collections at NLGRP)

situ approaches. Germplasm conserved ex situ includes traditional Native American landraces of corn, bean, and squash, as well as approximately 100 other species of crops and their wild relatives. The NS/S collection includes over 180 accessions of CWR in the genera *Amaranthus* L., *Chenopodium* L., *Capsicum*, *Cucurbita*, *Tagetes* L., *Gossypium* L., *Helianthus* L., *Carthamus* L., *Atriplex* L., *Nicotiana*, *Phaseolus*, *Proboscidea* Schmidel, *Physalis* L., *Salvia* L., and *Zea* L. collected from the southwestern USA and northwestern Mexico (Laura Jones, personal communication, 3 August 2016). The samples were reported to be of varying viability and availability.

4.3.3.3 Botanic Gardens Conservation International (BGCI)

The US office (https://www.bgci.org/usa/) for the BGCI is housed at the Chicago Botanic Garden (CBG). The BGCI's Global Seed Conservation Challenge is an initiative that promotes the participation of botanic gardens in seed banking. According to BGCI records, sixty-two botanic gardens in the USA are already banking seed for conservation. For example, the CBG manages the Dixon National Tallgrass Prairie Seed Bank (https://www.chicagobotanic.org/research/conservation_and_restoration/seed_banking), which preserves germplasm of native plant species, including some CWR, from the Upper Midwest and provides seed to requestors for research and restoration. Accessions can be searched in the CBG Science Collections database (http://sciencecollections.org/content/search-collections) and requests emailed to the Seed Bank. As noted earlier in the chapter, the North American Botanic Garden Strategy for Plant Conservation 2016–2020 (BGCI 2016) specifically includes the target of conservation of CWR through both in situ and ex situ means.

4.3.3.4 Plant Collections Network (PCN)

Members of the American Public Gardens Association (APGA) include many of the US botanical gardens and arboreta that maintain and conduct research on collections of germplasm of ornamental plants. Beginning in the early 1990s, the APGA collaborated with USDA-ARS to develop the PCN (https://publicgardens.org/programs/about-plant-collections-network), with the goals of developing a continent-wide approach to preservation of woody and herbaceous plant germplasm and promoting professional standards of plant collections management. In conjunction with the USDA-ARS National Arboretum, the APGA coordinates the PCN, which today includes 74 participating gardens and arboreta and 125 nationally accredited plant collections (Meyer 2017). The collections are curated using defined practices for documentation and maintenance, and germplasm is made available by the responsible institutions for research and breeding. Many of the plant collections include or are composed exclusively of species of native North American genera important for ornamental or landscape use (e.g., *Agave* L., *Cercis* L., *Cornus* L., *Gymnocladus* Lam., *Kalmia* L., *Magnolia* L., *Quercus* L., *Rhododendron* L., *Stewartia* L. Lawson, *Tilia* L., *Trillium* L., and *Ulmus* L.).

4.3.3.5 North Carolina Arboretum Germplasm Repository

The Repository (http://www.ncarboretum.org/impact/germplasm-repository/), located at the North Carolina Arboretum in Asheville, maintains over 2000 samples of germplasm of native plants, mostly from western North Carolina. Documentation of germplasm with voucher specimens and associated passport data is a key feature of the collection. The collection includes native medicinal germplasm including

American ginseng (*Panax quinquefolius*), black cohosh (*Actaea racemosa*), and goldenseal (*Hydrastis canadensis*). Germplasm is distributed for collaborative research on crop improvement and development of new crops and commodities for the natural product industry in North Carolina. The Repository also maintains a fungal endophyte germplasm collection.

4.4 In Situ Conservation

Bretting and Duvick (1997) stressed the dynamic character of in situ conservation, in which new traits and combinations of traits continue to evolve in response to abiotic and biotic changes in the environment. The in situ conservation of CWR depends on the protection and management of the natural or seminatural habitats in which they occur. In the USA, in situ conservation of CWR mostly occurs fortuitously on lands managed by a wide range of governmental and private entities. Protection of these lands ranges from wilderness areas that are to remain in their natural condition to areas in which commercial exploitation is allowed or even encouraged. It is usually not possible to easily assess which species or populations of CWR are conserved in these areas because of the rarity of biological inventories. Partly as a consequence of this lack of documentation, there are few targeted efforts in place for the monitoring and management that would favor their continued survival.

CWR in the USA are usually conserved in situ under the general umbrella of biodiversity. The current framework in the USA for managing and conserving biodiversity consists of both governmental and private programs to reduce habitat loss, establish protected areas, protect endangered species, restore degraded habitats, control nonnative species, collect and conserve germplasm, and others.

A survey of existing protected areas is the logical starting point for evaluating the current status and potential for conservation of CWR in situ. The USA maintains a complicated mix of protected areas under the control of federal, state, and local governments, as well as private landowners (Fig. 4.5). The contribution of each sector varies widely in different regions of the country. The area west of the Mississippi River is dominated by federally owned lands, followed in order by lands under the control of state, regional, and local ownership, Native American lands, and privately held lands. Protected lands east of the Mississippi are more commonly under state, regional, and local ownership and have a greater contribution from private nonprofit organizations and individuals than in the west. Only 9% of the protected acreage in the USA is east of the Mississippi (Meyer et al. 2012). The focus of national discussions on biodiversity conservation has often emphasized federal management of public lands and protection of endangered and threatened species. However, state, local, and private lands are also critical for a more comprehensive conservation of US CWR, especially as the highest concentration of prioritized species occurs east of the Mississippi, and provide many opportunities for conservation.

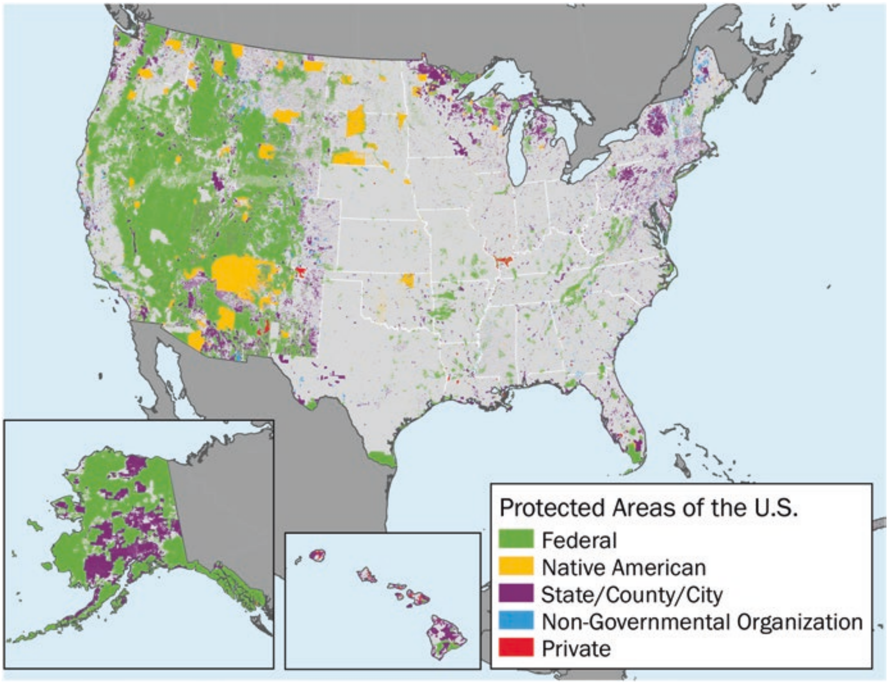


Fig. 4.5 Protected areas in the USA. Based on data downloaded from the Protected Areas Database (PAD-US) (USGS, GAP 2016)

Some conservation efforts in the USA involve comprehensive management of multiple species and protected areas administered by a variety of agencies. For example, in California, the Western Riverside County Regional Conservation Authority Multiple Species Habitat Conservation Plan was developed to protect 146 native species of rare, threatened, and endangered plants, birds, and animals and preserve 500,000 acres of their habitats (http://wrc-rca.org/about-rca/multiple-species-habitat-conservation-plan/) in a system of conserved lands. Partners in the plan include the Department of the Interior (DOI), the USFWS, the BLM, the California Resources Agency, the California Department of Fish and Wildlife, the Riverside County Habitat Conservation Agency, the county of Riverside, and several cities. *Juglans californica* S. Watson, the California black walnut, is among the plant species covered by the plan. *Allium munzii*, Munz's onion, a species listed as endangered under the federal Endangered Species Act and as threatened under the California Endangered Species Act, is covered as a narrow endemic. Monitoring and management activities are outlined for each of the covered species.

Nearly 20 years ago, four in situ conservation sites were established in the USA for the native rock grape (*Vitis rupestris* Scheele) (Pavek et al. 2001). The sites were recommended to land managers by ARS scientists who had evaluated morphological and genetic variation, population size and stability, and landholder commitment for populations across the range of the species. The rock grape was once distributed over 10 states from Texas through Arkansas and Missouri and east to Pennsylvania, but was considered to be rare and local throughout its range by the Nature Conservancy at the time of the study. In situ sites were established at two locations in the Wichita Mountains Wildlife Refuge, Oklahoma, one in the Clifty Creek State Natural History Area in Missouri, and along one stream in the Ouachita National Forest, Oklahoma. The current Ouachita National Forest Plan includes management guidelines and directs monitoring of the viability of the species along the original designated stream, as well as along two others (USDA, FSSR 2005). The status of the other three sites is not known. This example demonstrates the possibilities for in situ conservation on lands managed by different types of agencies, with the current awareness that follow-up and monitoring are necessary to maintain their full value.

The following section presents an introduction to the lands managed and protections applied by federal, state, county, local, tribal, and private entities.

4.4.1 National Government Agencies

4.4.1.1 Land Holdings

The federal government in the USA owns approximately 640 million acres, roughly 28% of the land area in the country (Vincent et al. 2014). These lands became part of the federal portfolio for a number of different reasons, none of which include selection based on the presence of CWR. Four federal agencies (see below) manage the largest proportion of federal lands, over 90% of which are located in the 11 westernmost states and Alaska (McKinney and Harmon 2004). The objectives of the agencies for land management vary, with the preservation of biodiversity receiving different amounts of emphasis.

The BLM manages 247.3 million acres, more than any other federal agency; most of this land is in the western USA. The BLM multiple-use mission, laid out in the Federal Land Policy and Management Act of 1976, includes outdoor recreation, domestic livestock grazing, energy and minerals development, timber production, fish and wildlife conservation, and conservation of natural, historical, cultural, and other resources. The BLM National Conservation Lands are designated for special management based on their conservation values and include National Conservation Areas, Wilderness Areas, National Wild and Scenic Rivers, Outstanding Natural Areas, and others.

The USFS manages 192.9 million acres as national forests and grasslands in 43 states and Puerto Rico. The 1976 National Forest Management Act requires FS lands to be managed for multiple uses, including recreation, grazing, timber, watershed protection, wildlife and fish, and wilderness and that all these uses must be systematically considered in developing forest plans.

The USFWS is responsible for over 89 million acres of lands and waters in the National Wildlife Refuge System (NWRS), not including the large marine national monuments (USFWS 2015). The NWRS, established in 1966, now includes more than 560 refuges and 38 wetland management districts. The primary purpose for including lands and waters in the system is their conservation and management and restoration of fish, wildlife, and plant resources and their habitats when necessary (https://www.fws.gov/refuges/about/mission.html). A large percentage of the total refuge system is located in Alaska, with 18% of the state designated as National Wildlife Refuges. Only about 2% of the total NWRS is located in the lower 48 states. Within the NWRS, Special Management Areas are designated, including wilderness, research natural areas, wild and scenic rivers, and wetlands of international importance (Ramsar sites). Five units of the refuge system are included in biosphere reserves (https://www.fws.gov/refuges/whm/specialMgmtArea.html).

The National Park Service (NPS) manages over 84 million acres that include over 400 national parks, national monuments, national seashores, national rivers, and other landmarks of conservation, cultural, scenic, or historical interest in the National Park System (https://www.nps.gov/aboutus/upload/NPS-Overview-02-09-17.pdf). Established by the Organic Act in 1916, the main purpose of the NPS is to preserve the natural and cultural elements of the National Park System unimpaired for the enjoyment of the current and future generations. In addition to the sites in the system, the NPS manages affiliated sites such as National Heritage Ares, National Wild and Scenic Rivers, National Historic Landmarks, and National Trails.

The Department of Defense (DOD), the Department of Energy (DOE), and the Tennessee Valley Authority (TVA) also manage large parcels of protected land. The DOD manages 14.4 million acres of military reservations in the USA and its territories (Vincent et al. 2014). The DOE manages 2.4 million acres across the country. The TVA, a self-funded government agency, manages approximately 765,000 acres, including the waters of its reservoir system (Tennessee Valley Authority 2011).

4.4.1.2 Categories of Protection

Legal protection aimed at safeguarding biodiversity in the USA may be applied to lands or to specific species. A number of laws and regulations in the USA protect public lands and biodiversity, including the National Environmental Policy Act 1969, the Renewable Resources Planning Act, the National Forest Management Act, the Federal Lands Protection and Management Act of 1976, and the Endangered Species Act of 1973. Permanently protected lands have the most potential for serving the purpose of long-term in situ reserves for CWR.

4.4.1.2.1 Protection for Federal Lands

There are various levels of legal protection afforded to federal lands. Some designations for protected areas are restricted to use by one agency, while others are authorized for use by multiple agencies. Some of the designations can be applied by the

Table 4.5 Designated wilderness areas managed by federal agencies

Agency	Wilderness area (million acres)	Number of wilderness areas
National Park Service	44	61
US Forest Service	36.6	445
US Fish & Wildlife Service	20	71
Bureau of Land Management	8.7	224

agency managing the land and others only by Congress or the President. Each carries with it a unique set of management regulations. The main designations for protected areas are described below.

Wilderness is the highest level of protection available for federal lands. The Wilderness Act of 1964 established the National Wilderness Preservation System and defined wilderness as “an area of undeveloped federal land” that is to be “protected and managed so as to preserve its natural conditions.” It immediately protected 9.1 million acres of undeveloped federal land and set up a system of adding new areas, which are designated by Congress through legislation. As of 2017, over 109 million acres in more than 800 units of federal lands in 44 states have been designated as wilderness (http://www.wilderness.net/factsheet.cfm) managed by four agencies: NPS, USFS, USFWS, and BLM (Table 4.5 and Fig. 4.6). Most wilderness areas are concentrated in the western USA, with Alaska, California, Arizona, Idaho, and Washington topping the list of states with the largest areas. Wilderness areas in the eastern USA are much less extensive, with large wilderness areas occurring only in Florida and Minnesota.

Research Natural Areas (RNAs) are part of a national network of sites placed under permanent protection to maintain biological diversity and provide ecological baseline information, education, and research. Research must not alter existing conditions. The RNAs usually are composed of undisturbed hydrogeologic and biotic community types between 100 and 10,000 acres in size. Several federal agencies, including the USFS, NPS, BLM, USFWS, and DOE, manage RNAs. The USFS has designated over 570,000 acres in more than 450 RNAs on 175 USFS lands. BLM considers the RNAs as a type of Area of Critical Environmental Concern (see description below).

National conservation areas are areas of any size with scientific, cultural, historical, or recreational features managed by the BLM. They must be designated by Congress.

National monuments are locations of natural significance, Native American sites, or other historic sites managed by eight federal agencies. There are no restrictions on the size of these areas. They can be established by Presidential Proclamation or Congressional legislation.

Botanical areas are areas with rare plants or unique plant communities that may be designated by several federal agencies (Fig. 4.7). They are managed to emphasize conservation, but other uses that do not impact the botanical resources are allowed. Land Management Plans specify how botanical areas are to be managed.

Fig. 4.6 The La Madre Mountain Wilderness in Nevada, managed by the BLM and USFS. (Photo: K.A. Williams)

Areas of critical environmental concern are BLM lands of any size that are put under special management to protect and prevent damage to historic, natural, or scenic values. They are designated by BLM through the land use planning process. Most have been designated based on botanical features, including unique or outstanding plant communities.

Biosphere reserves are protected areas that are internationally recognized by the United Nations Educational, Scientific and Cultural Organization (UNESCO) Man and the Biosphere Program. Nearly 50 protected areas in the USA have been designated as biosphere reserves. Biosphere reserves are nominated by national governments for this designation, but remain under the sovereign control of the country where they are located.

Fig. 4.7 Cranberry Glades Botanical Area, Monongahela National Forest, West Virginia. Large populations of the large cranberry (*Vaccinium macrocarpon* Aiton) and the small cranberry (*V. oxycoccos* L.) occur here. (Photo: K.A. Williams)

Wild Chile Botanical Area

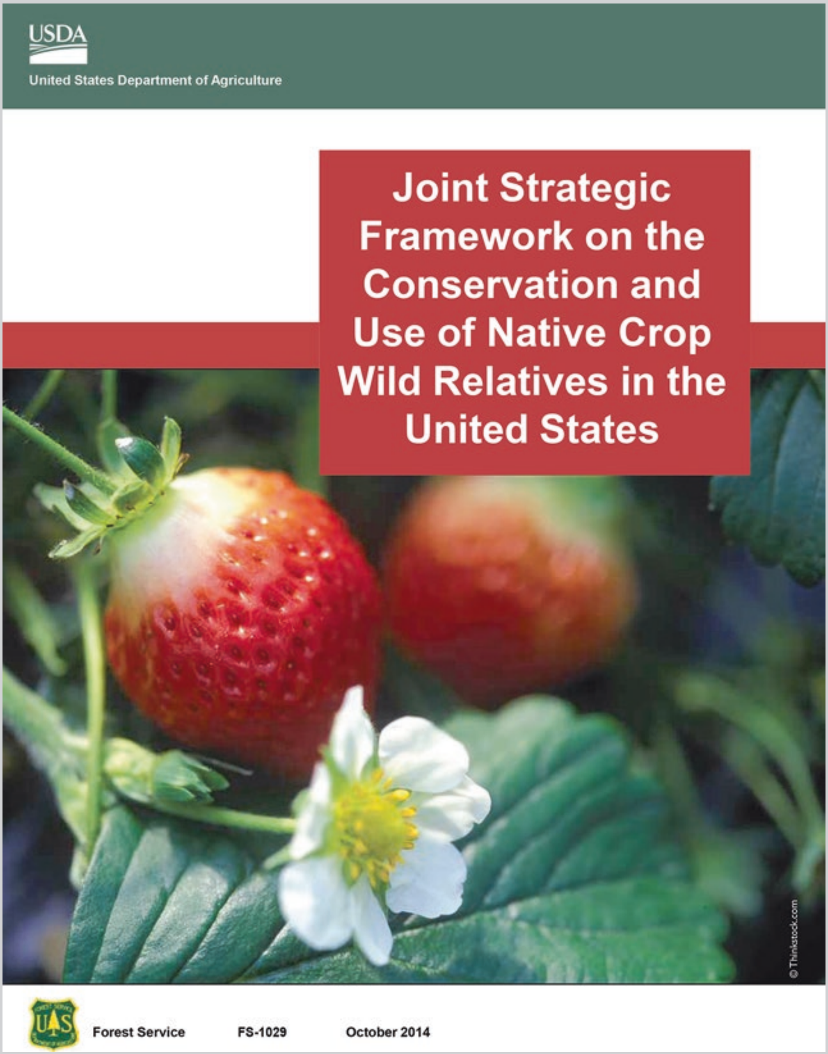
The Wild Chile Botanical Area, in the Rock Corral Canyon subwatershed of the Coronado National Forest in southeastern Arizona, is a 2836 acre area designated in 1999 to bring attention and provide protection and research opportunities for the wild chile (*Capsicum annuum* L. var. *glabriusculum* (Dunal) Heiser & Pickersgill) and other plants of economic importance or conservation concern. This was the first botanical area set aside to protect the wild relatives of crops. Native SEEDS/SEARCH (see Sect. 4.3.3.2) brought attention to the importance of the area and collaborated with the USFS in the establishment of the botanical area. For centuries, the Tohono O'odham have harvested the wild chiles from this area, which is at the northern limit for the species (Nabhan 1990a). According to the Coronado National Forest Draft Land and Resource Management Plan (USFS 2013), wild cotton, tepary beans, and two species of wild gourds are also found in the Wild Chile Botanical Area. The management plan allows for traditional harvesting of the chiles after receipt of a special use permit and with the requirement that the existing populations are not harmed.

4.4.1.2.2 Protection for Species

No special protections for CWR are provided by any federal agencies. Crop wild relative species that are threatened or endangered are treated as any other plant under the Endangered Species Act (ESA) of 1973 (USFWS 1973), which was established to protect critically imperiled species, including plants, and their habitat in the USA. The ESA is administered by the USFWS and the Commerce Department's National Marine Fisheries Service (NMFS). In order to gain protection under the ESA, a species must be listed as either threatened or endangered by the USFWS or the NMFS. It requires designation of "critical habitat" essential for the species' survival and development of recovery plans. The ESA requires federal agencies to ensure that their activities do not jeopardize the existence of listed species of plants and animals or adversely modify designated critical habitat. The ESA also encourages the cooperation of the federal government with states and provides for financial assistance to the states to assist in development of programs for the conservation of endangered or threatened species.

All federal agencies are required by law to sustain and protect rare and endangered species on lands under their management. Species at risk on National Forest System lands are managed through the National Threatened, Endangered, and Sensitive Species (TES) Program. The Program supports recovery and conservation of threatened, endangered, and sensitive species and their habitats. They coordinate with the Botany and Rare Plant Programs, as well as other USFS resource management programs. The BLM Threatened and Endangered Species Program seeks to recover federally listed species and to conserve other non-listed rare plants. The Biological Resources Division of the NPS works with individual parks on the monitoring of threatened and endangered species and efforts to assist in their recovery. The USFWS with its singular focus on conservation and restoration of biological resources has the most direct connection to the ESA. Many wildlife refuges were established specifically to protect endangered species, and the agency also works with owners of surrounding land to restore and recover federally listed threatened or endangered species. At least three species ranked as Priority 1A or 1B (*Helianthus paradoxus* Heiser, *Manihot walkerae* Croizat, and *Prunus geniculata* R. M. Harper) in the national inventory of the USA (Khoury et al. 2013) are documented as being among the endangered or threatened CWR occurring on National Wildlife Refuges (USFWS 2017). The DOD, as required by the Sikes Act of 1960, develops and implements plans for conservation and rehabilitation of significant natural resources on military installations. As part of this effort, the DOD cooperates with the USFWS and the NMFS to manage endangered species on military lands. Approximately 233 threatened and endangered plants had been included in DOD management plans as of 2015 (Department of Defense Natural Resources Program 2016).

USFS-ARS Collaboration on CWR Conservation



USDA
United States Department of Agriculture

Joint Strategic Framework on the Conservation and Use of Native Crop Wild Relatives in the United States

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Forest Service FS-1029 October 2014

Fig. 4.8 The USFS-ARS Strategic Framework on Conservation of CWR

A recent collaboration between two sister agencies within the USDA, the USFS and the ARS, seeks to harness the complementary strengths of the two agencies to support the conservation of CWR. The USFS focus on in situ conservation and the ARS focus on ex situ conservation make these agencies logical partners in the complementary conservation of CWR in the USA, specifically on lands in the National Forest System. The collaboration was formalized through an agreement between the agencies and further developed in the *USFS-ARS Joint Strategic Framework on the Conservation and Use of Native Crop Wild Relatives in the United States* (US Forest Service 2014, Fig. 4.8). The foundation of the strategic framework is its emphasis on complementary conservation, with plants in living populations on National Forest Lands linked with germplasm conserved ex situ in genebanks of the NPGS. Two general approaches are established, one focusing on conserving the CWR of one specific crop and the other on CWR of multiple crops within the boundaries of a specific protected area.

A pilot study for the first approach to complementary conservation in the Framework is evaluating the wild relatives of cranberry: the large cranberry (*Vaccinium macrocarpon*) and the small cranberry (*Vaccinium oxycoccos*). Populations of these species are being studied on National Forests and will be prioritized for designation as *In Situ Genetic Resource Reserves* (IGRRs). This designation will be based on location, distance from other populations, sustainability, population size, genetic profile, ease of access, and cultural significance to Native Americans. Long-term management plans will be implemented by the USFS to monitor, manage, and safeguard the security of the populations. Representative germplasm will be maintained as seedlots and plants by the NPGS at the National Clonal Germplasm Repository in Corvallis, Oregon. In the future, expansion of the study to populations outside the National Forest System is planned to encompass broader genetic diversity of the wild cranberry species.

4.4.2 Tribal Lands

The Department of the Interior's Bureau of Indian Affairs (BIA) is responsible for the administration and management of 55 million acres held in trust by the US government for 567 federally recognized American Indian tribes and Alaska Natives (https://www.bia.gov/about-us). These lands fall under tribal government authority, are not considered federal lands, and are not subject to state laws. There is a limit on how the lands can be used, and federal approval is required for some actions. Many of these heterogeneous lands are undeveloped and house a reservoir of natural ecosystems and species, including CWR. A number of tribal governments are actively involved in biodiversity conservation, but there has been little coordination of conservation efforts on these lands with other efforts across the country (Defenders of Wildlife 2008).

Some tribal governments have designated wilderness areas. An outstanding example is the Mission Mountains Tribal Wilderness in Montana, an area of over 90,000 acres designated by the Confederated Salish and Kootenai tribes in 1979. It abuts the Mission Mountains Wilderness of the Flathead National Forest.

4.4.3 State/County/Local Government Agencies

4.4.3.1 Land Holdings

States, counties, townships, metropolitan authorities, and other local governmental entities throughout the USA manage thousands of protected areas. State park programs managed by state governments first began to develop in the late nineteenth century. Their development was greatly augmented by the Civilian Conservation Corps (CCC), a Federal work relief program, which from 1933 through the 1940s helped develop the infrastructure for parks in many states (Walls 2009). States such as Florida established parks to take advantage of the support provided by the CCC. The distribution of acreage and number of state park sites vary widely across states (Walls 2009). Alaska has the most acreage in parks, and New York has the greatest number of parks. In the continental USA, land conservation efforts at this level have generally been more common in the eastern USA (Meyer et al. 2012). Parks range from small urban parks to large parks such as the 6 million acre Adirondack Park that includes both public and private lands in New York. Many states also manage game and recreation areas, within the confines of which are found many CWR.

4.4.3.2 Categories of Protection

Some state governments have designated wilderness areas under state authority. Each of these states has distinct requirements for designation and regulations for management. Over 45% of state-owned part of the Adirondack Park in New York is designated as wilderness.

The ESA recognized the important role of states in protecting endangered species and authorized the DOI to enter into cooperative agreements with states to establish programs to strengthen state efforts. However, the ESA provides little protection for plants occurring on nonfederal land, in contrast to the protections for animals (Havens et al. 2014). Plant protection depends on state endangered species laws. Most states now have these laws, but they are highly diverse in their coverage, ranging from the prohibition of harming an endangered species to complete plans for listing, management, and protection (George and Snape 2010). Two states, West Virginia and Wyoming, do not have laws to protect endangered species, and the laws in 17 states do not protect any endangered or threatened plants (Camacho et al. 2017). Laws in only 18 states cover all the animals and plants covered by the ESA (Camacho et al. 2017). As far as can be determined, no states offer special designation or protection to CWR. When a species that is a CWR is listed, it is treated the same as other listed species.

4.4.4 Private Landholders/Organizations

4.4.4.1 Land Holdings

The importance of private nonprofit organizations and individual owners to conservation is underscored by the fact that private property accounts for over 90% of the land area in some states, including Illinois and Texas (Defenders of Wildlife 2008). Many private lands have higher levels of species diversity because they are located on moderately to highly productive soils and at lower elevations than public lands. A large number of these ecosystems, including low elevation grasslands and shrublands in the central USA, are poorly represented on public lands (Aycrigg et al. 2013). The total extent of privately protected lands and coverage of ecosystems are not known as they have not been comprehensively documented (Riordan 2009).

The contribution of private investment extends beyond the creation of privately owned parks. In several cases, individual philanthropists have purchased lands that were later donated to government agencies and became public parks (e.g., private donations of land helped to create the Acadia National Park in Maine).

4.4.4.2 Conservation Easements

A conservation easement is a legally binding agreement between a landowner and a land trust or a government agency that limits the use of the land to protect its conservation value, while still leaving the land in private ownership. Conservation easements are becoming very common as a tool to preserve private lands (Meyer et al. 2012).

4.4.4.3 Land Trusts

At the state and local levels, private conservation land trusts, or land conservancies, are increasingly being used as a means to preserve natural areas, open space, farmland, ranchland, historical sites, and other resources. These trusts may acquire land outright, purchase conservation easements to prevent development, or purchase mineral or other rights. According to a 2015 census conducted by the Land Trust Alliance (2015), acreage in the USA that was protected with the help of state, local, and national land trusts amounted to over 56 million acres, more than double the amount of land in national parks in the lower 48 states.

4.4.4.4 The Nature Conservancy (TNC)

One of the most influential nongovernmental organizations involved with land preservation in the USA is the TNC (http://www.nature.org/). The TNC has helped to protect more than 21 million acres in the country (http://www.nature.org/about-us/

private-lands-conservation/) through various means. The organization owns almost 2 million acres and has more than 3 million acres of conservation easements. Other land protection strategies used by TNC are management agreements and leases and the promotion of acquisition and protection of ecologically valuable lands by private individuals. The TNC purchases land of priority conservation areas or buffer zones around natural areas and later sells the land to private buyers willing to ensure conservation of the land through a conservation easement.

4.4.4.5 Categories of Protection

As previously discussed (see Sect. 4.4.3), the ESA does not apply to plants on non-federal land unless federal permits are required or significant federal funding is received ((Camacho et al. 2017). Consequently, protection of plants on private lands depends on state endangered species laws, which are diverse in coverage and often very limited in their scope.

4.5 Information Resources for CWR and WUS

A number of additional sources not already mentioned contribute useful information and tools relevant to the conservation and use of CWR and WUS in the USA. These sources include basic information on the taxonomy, characteristics, locations of populations, and conservation status that may be used to prioritize and plan germplasm collections and to assess and establish in situ reserves.

4.5.1 NatureServe

NatureServe supports a public-private network of biodiversity inventory programs throughout the Western Hemisphere, including those in all 50 states, known as natural heritage programs (http://explorer.natureserve.org/). These programs help to provide the scientific knowledge that forms the basis for effective conservation action (Kareiva et al. 2014). NatureServe has also developed several tools for conservation planning, two of which are described below.

> **NatureServe Explorer** (www.natureserve.org/explorer) is a comprehensive publically available database on species and ecosystems, including location records from the network programs, reporting the condition and distribution of species and ecosystems in the USA and Canada. Detailed information is available on rare and endangered species and threatened ecosystems, and conservation status assessments are provided across species entire ranges and also within each state, with the conservation status rank ranging from presumed extinct (GX) to secure (G5). The data on species abundance and threat status is much more extensive than that offered by the IUCN Red List (Havens et al. 2014). Information is available in the database for many CWR taxa.

LandScope America (http://www.landscope.org/connect/find/partners/natureserve/) is an online tool developed by NatureServe, the National Geographic Society, and other institutions to provide resources to support informed conservation. It combines written, mapped, and multimedia content on conservation priorities, protected areas, threats, plants and animals, and ecosystems, which can be applied to the implementation of in situ conservation for CWR.

4.5.2 Flora of North America (FNA)

The FNA (FNA Editorial Committee, eds. 1993; http://floranorthamerica.org/) provides information on the taxonomy, distribution, and morphological characteristics of native and naturalized plants in the USA and Canada. It includes identification keys, distribution maps, illustrations, uses, phenology, ethnobotanical uses, and other information. The information is also available in the Tropicos database (http://www.tropicos.org/) of the Missouri Botanical Garden. Herbarium specimens are the sources of much of the information reported. When complete, the FNA will have 30 volumes. The finished volumes are available both in print and online (http://www.efloras.org/flora_page.aspx?flora_id=1).

4.5.3 The Biota of North America Program (BONAP)

The BONAP (http://www.bonap.org/) is an online database with information on taxonomy, distribution, biological attributes, conservation status, images, and occurrences of native and naturalized species of plants, in the USA and Canada. Included within the database are many plants that are CWR.

4.5.4 Botanic Garden Conservation International (BGCI)

The BGCI's PlantSearch (http://www.bgci.org/plant_search.php) allows users to search living collections at contributing institutions. Searches can be done for plants identified as CWR in 68 genera, and germplasm of specific taxa can be requested. The online information does not include details on individual accessions or allow users to see which institutions hold the material, but does allow a general assessment of the inclusion of CWR germplasm in the ex situ collections with information in the database.

4.5.5 PLANTS Database

The PLANTS Database (https://plants.usda.gov/), managed by the USDA-NRCS, presents information about all types of plants in the USA and its territories. Information includes taxonomy, nativity, federal and state conservation status, invasiveness, images, and distribution maps, often to county level. For some plants, detailed plant guides are also provided. Many CWR are included in the database.

4.5.6 Herbaria

In addition to the essential role of herbarium specimens as reference material for identifying specimens and studying the characteristics of CWR species, data obtained from herbaria are useful for documenting the locations where they were collected, their abundance, and phenological traits. Increasingly herbaria are making specimen data available online (Havens et al. 2014). Examples of online databases with US CWR species include the Missouri Botanical Garden's Tropicos database (http://www.tropicos.org) and the Smithsonian National Museum of Natural History Department of Botany Herbarium (http://collections.nmnh.si.edu/search/botany). Some regional consortia in the USA, such as the Southwestern Environmental Information Network (SEINet; http://swbiodiversity.org/seinet/index.php) and the Consortium of Pacific North West Herbaria (CPNWH; http://www.pnwherbaria.org/data/search.php) provide access to data from multiple sources and mapping tools to visualize them. Records from many herbaria, as well as other institutions around the world, are available from the Global Biodiversity Information Facility (GBIF; https://www.gbif.org/), which shares information about where and when biological organisms have been recorded.

4.5.7 Plant Inventories

Information on the occurrences of CWR is fundamental to planning for their conservation. Very few complete inventories of plants or other biodiversity have been conducted for federal or other protected areas. Checklists have been made available for some areas, such as some RNAs in National Forests (https://www.nrs.fs.fed.us/rna/established/). More information is available on the locations of populations of endangered and threatened species than for others, although they may be kept private to protect overexploitation. The All Taxa Biodiversity Inventory (ATBI), a project of the nonprofit Discover Life in America, is developing an inventory in the Great Smoky Mountains National Park (https://www.dlia.org/). A spinoff of this effort, the ATBI Alliance is organizing inventories in other national and state parks, including the Adirondack Park. These inventories will be useful to identify locations of populations of CWR from which germplasm may be collected for ex situ conservation and which may be considered as sites for in situ conservation.

4.6 General Assessment of Conservation of Native Wild CWR

Numerous CWR in the USA have already contributed to food security and economic development, both within the country and around the world. Great potential for expanded contributions exists if these resources are adequately conserved and utilized. However, the possibilities are currently limited by the underrepresentation of US CWR in germplasm collections, including the NPGS. Of the 29 crops listed in Table 4.3 with priority 1A CWR taxa native to the USA, 11 do not have even one representative sample for every related taxon. Moreover, some crops are lacking representation for several of the related CWR taxa (e.g., *Prunus*, *Rubus*, and *Vitis*). The CPC National Collection of Endangered Plants includes additional taxa of three of the Priority 1A taxa and also includes several Priority 1B CWR not conserved in the NPGS. In addition to the lack of representation of some taxa, most existing germplasm collections do not fully represent the diversity found throughout their geographic ranges in the USA or other countries in which they occur. In a study utilizing potential distribution modeling to assess geographic gaps in germplasm collections, Castañeda-Álvarez et al. (2016) found significant gaps in many CWR native to the USA. Detailed analysis of geographic gaps in germplasm collections of 36 sunflower CWR suggested that at least half were in dire need of additional collecting (Kantar et al. (2015), and an examination of gaps in genebank collections of sweet potato CWR identified the extreme southeastern USA as a high priority region for collection of underrepresented CWR (Khoury et al. 2015). Analysis of gaps and collection of germplasm are necessary to ensure that the full range of diversity in all crop genepools in the USA is conserved and available for use. For some taxa, additional research is necessary to develop conservation protocols that allow them to be efficiently conserved ex situ (e.g., *Zizania* spp.).

The adequacy of the conservation of CWR in situ in the USA is more difficult to assess than the ex situ coverage. Although the flora of the USA is well documented compared to many other countries (Havens et al. 2014), much relevant knowledge is lacking. More extensive data compilations and expanded surveys are needed to determine if or how well specific CWR are being conserved in existing protected areas. Detailed information on the locations of populations of CWR would facilitate both approaches provided for in the USFS-ARS Framework for Conservation of CWR – the designation of either individual populations of one CWR taxon or of areas with multiple taxa CWR as in situ reserves. Moreover, populations of some CWR likely do not occur in any existing protected areas, and the full geographic distribution of others will not be covered by these areas. In some cases, alternative arrangements, such as agreements on management with landowners, will be necessary to conserve these CWR. Even when CWR do occur in protected areas, they may not be adequately protected, and the varying management tools being employed may not be optimal for their long-term survival. In these cases, adjustments in management practices will necessarily be applied after designation as in situ reserves.

Many of the lands on which CWR occur are likely to include areas that are privately owned. Especially in the eastern USA with its low incidence of federal and state protected areas (Fig. 4.5), inclusion of private landowners will be necessary to adequately conserve populations of CWR. This is especially significant because the concentration of CWR taxa is highest in this area of the country (Fig. 4.2). Although preserves and conservation easements were not specifically designed for the conservation of CWR, they may be a viable option for regions with a low incidence of protected areas.

Protecting lands where CWR or other plants occur is not sufficient to ensure their long-term survival (Havens 2014). Crop wild relatives will continue to be threatened by the same factors that threaten other wild species, including invasive species, habitat fragmentation and degradation, and climate change. Several CWR taxa in the USA are recognized as endangered or threatened by the USFWS and listed under the Endangered Species Act (ESA, ECOS 2017), and additional CWR taxa have been categorized as having some level of conservation concern by NatureServe (2017). However, the full extent of the conservation status of these and other taxa is not completely known as inadequate resources have been devoted to their assessment. Also, the ESA only provides protections for plants located on federal lands or where federal funding is provided to other landowners, and protections vary widely on state lands (Havens 2014). The lack of protection for plants by laws in 19 states and the absence of restrictions on private land use in most states (Camacho 2017) are significant concerns.

Even for those taxa designated as endangered or threatened by the USFWS under the ESA or by states under their laws, protection does consider individual populations with unique traits. From an agricultural perspective, unique traits found at the population level are often of great value and warrant consideration for conservation. Research on the genetic diversity of populations within species will help to inform decisions on which populations should be prioritized for conservation.

The development and implementation of a comprehensive national program for in situ conservation of CWR is a challenging undertaking requiring consideration of many interacting factors. Construction of a program requires the identification of the appropriate species, populations, and locations, collaboration between land management agencies, and development of management and monitoring practices for species with widely varying adaptations. Hurdles to overcome include the different regulations and structures in the multiple land-holding agencies, the absence of some CWR in current protected areas, and the competing pressures for land use. In addition, many CWR thrive in disturbed environments, such the naturally disrupted areas created by wildfires, floods, or avalanche, or in human-induced disruptions, such as at the edges of agricultural fields. Such CWR require different plans to ensure their survival than those in more stable environments.

4.7 Conclusion: Challenges and Opportunities to Conserve CWR in the USA

The foundation for the adequate conservation of CWR in the USA is expanding, but much work remains to devise and implement complementary strategies incorporating both ex situ and in situ conservation. Necessary elements include the detailed analysis of the current status of diversity and conservation of CWR ex situ and in situ, the collection and conservation ex situ of CWR of high priority, designation and support of protected areas as in situ reserves in areas of high or significant diversity, and efforts to increase awareness of the importance of CWR conservation. Successful achievement of these objectives will require partnerships among institutions, tribal governments, governmental agencies, and countries, innovative ideas on how to conserve this diversity, and the commitment to conserve.

Fortunately, awareness of and efforts to conserve CWR in the USA and elsewhere are increasing. Inclusion of CWR in the strategies and frameworks for biodiversity conservation in the USA has recently become more common (e.g., PCA National Framework for Progress; National Seed Strategy for Rehabilitation and Restoration; North American Botanic Garden Strategy for Plant Conservation 2016–2020). Articles and books are helping to communicate to the public knowledge about the realized and potential contributions of CWR and native crops (Fisher 2012; Harvey 2017; Mark 2017; Moore 2015) an inventory of CWR in the USA (Khoury et al. 2013), the first vital step in comprehensive conservation planning, has been developed. Experts in assessing and filling gaps in germplasm collections are pursuing explorations for the CWR of many crops, with substantial progress already made in the comprehensive collection of some groups of CWR (e.g., potato [Bamberg et al. 2016], small fruits (Chap. 20, this book), and sunflower [Marek 2016; Seiler et al. 2017; Chap. 25, this book).

In addition, programs of many institutions with varying priorities are already focused on CWR conservation, especially ex situ conservation. Improved linkages between these programs show promise to further fill gaps in germplasm collections and advance the conservation of CWR in situ. The SOS program managed by the BLM is collecting native plant taxa from wild populations, from which samples are deposited in the NPGS. Additional cooperation between the NPGS and SOS could expand the collection of CWR. Private gardens in the CPC network are active in ex situ conservation and reintroduction of rare plants, often in partnership with land management agencies (Havens et al. 2014); some of these plants include those that are CWR. A partnership between the USFS and ARS for complementary conservation of CWR, with a pilot project on cranberry CWR, provides a model for collaboration between institutions for linking ex situ and in situ conservation. The observation that some CWR are restricted in occurrence to private lands highlights the need to develop new models of collaboration between public and private entities to further conservation goals.

Going beyond multiparty collaborations within the USA, comprehensive conservation of CWR will necessarily involve collaboration with the bordering countries of Mexico and Canada. Although the geographic distributions of certain CWR are restricted to only one country in North America, there are few crops in which the entire genepool does not cross borders.

Much of the research needed to better understand the distributions, storage behavior, and genetic diversity present in CWR will also inform improved conservation both ex situ and in situ. Although many CWR are believed to be conserved in protected areas, most populations are not documented. Inventories to elucidate the occurrence of CWR in protected areas would advance our capacity to better understand how completely these taxa are conserved throughout their ranges, enable monitoring of threats to their survival in the future, and open possibilities for collecting germplasm for ex situ conservation.

As a way forward, a comprehensive national CWR initiative with multiagency partnership and collaboration is needed to:

- Conduct a comprehensive assessment of CWR in the USA to define the highest priority species, understand their distribution and patterns of diversity, and identify gaps in their conservation, both in genebanks and in their natural habitats.
- Systematically collect prioritized crop wild relatives across the USA.
- Process collected samples and associated information for genebank conservation and distribution.
- Designate habitat conservation sites for populations of the highest priority CWR in existing protected areas, and advocate for the designation of additional protected areas, as needed.
- Collaborate on educational and communications initiatives to help raise awareness and provide a backdrop for ongoing support of CWR genetic resource conservation.

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Chapter 5
Working with Indigenous Communities: The Original Caretakers of Crops and Crop Wild Relatives

Scott M. Herron

Abstract Indigenous peoples are numerous, both in population and the number of distinct cultures across North America. They have managed plants in natural and agricultural environments for thousands of years in North America. Many of these domesticated, cultivated, and wild plants remain important within the indigenous cultures and across the globe. In order for these resources to be used to their full potential, there is a need for cooperative governance of the plants as well as the need to treat each tribe/First Nation as an individual government entity. Select case studies from the Northern Great Lakes region illustrate the ongoing natural resource management by tribal/First Nation governments in an effort to demonstrate strategies that researchers might employ to achieve productive working relationships with these original caretakers of crops and crop wild relatives.

Keywords Governance · Epistemology · Cooperation · Natural resource management · Tribal · First Nations · indigenous

5.1 Introduction

Wild plants and wild-crafted plants that later were domesticated into crops all have shared a landscape with North America's indigenous communities for 1000 and 10,000 years (Kraft et al. 2014; Smith 1998). Prior to the agricultural revolution, the traditional human diet was composed of collected wild plants and mushrooms (their edible and medicinal parts), gathered fish, and hunted small and large game.

S. M. Herron (✉)
Department of Biological Sciences, Ferris State University, Big Rapids, MI, USA
e-mail: scottherron@ferris.edu

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It is clear from working for and within indigenous communities that a land management policy focused on sustainability was incorporated into the historic landscape across North America in precolonial times and, to the extent possible, in modern times. In recent decades, co-management with colonial settler governments through federal, state/provincial, and/or local agencies is often imposed upon, and sometimes welcomed, by indigenous communities in Canada, the United States, and Mexico (Busiahn and Gilbert 2009; Intergovernmental Policy Council 2007; Kenney 2012; Washington Department of Fish and Wildlife 2017). Often, differing worldviews put indigenous management strategies at odds with Christian-influenced, scientifically guided management policies of such government agencies (Busiahn and Gilbert 2009; Kenney 2012).

5.2 Epistemological Differences

Philosophically most indigenous communities and individuals perceive the land, waterbodies, sky, and weather as animate beings, akin to living relatives, referring to them verbally as *mother earth*, *father sky*, *grandmother moon*, *thunderbeings*, etc. Alternatively, most descendants of colonial settlers are influenced more by Judeo-Christian worldviews and do not recognize the intimate familial relationships between the people of this landscape and the landscape itself, which includes plants. This difference has resulted in the current populations of crop wild relatives and wild plants being products of two distinct cultural relationships with the plant world. In the indigenous relationship, plants are relatives, and in the viewpoint of colonial settlers, plants are nature's bounty to be studied, harvested, and used however we as "God's chosen species" see fit (Deloria 1994; Kenney 2012; Geniusz 2015).

5.3 Historical Legacy and Implications for Collaboration

Today, when a scientist or natural resource manager attempts to work with one or more indigenous communities to address an issue, such as how to maintain crop wild relative populations, genetic diversity, or how to address the accumulation of toxic chemical pollutants, they often lack the cultural knowledge and communication strategies to succeed. This chapter will attempt to provide some essential information to assist all invested individuals seeking to build mutually respectful, cross-cultural relationships. It is important to recognize that native/indigenous communities are not homogenous. In the United States, there are 566 federally recognized tribal nations and 62 tribal nations that are recognized by states. In Canada there are 634 recognized First Nation groups. Mexico has recognized at least 62 different language groups, and about 20% of the population has indigenous ancestry.

The differences between indigenous and colonial settler histories, both politically and culturally, have led to divergent ways of understanding the world. It is important to recognize that plant communities did not acquire their precolonial settler status independent of human intervention. In fact, as many of the authors in this book will explain, indigenous groups across North America played important roles in changing the physical and genetic characteristics of plants by cultivating many and fully domesticating others into forms readers are familiar with today. Once these indigenous human-plant interactions are fully recognized, further acknowledgment of the historical cultural practices that have kept domesticated plants, their crop wild relatives, and the other wild plants on the landscape in a healthy condition must be granted to local Native American, First Nation, and indigenous communities.

Across North America there are a wide range of tribal and US federal agencies that interact to manage the conservation of plants and animals. For example, in Southeastern Great Lakes region, some of the only existing communities of rare tallgrass prairie, wet meadows, Carolinian forests, and oak savannahs exist on Walpole Island First Nation, also known as *Bkejwanong* in the Anishinaabe language (Jacobs et al. 2002). Three of the four plant communities listed have historically been intentionally burned by tribal members and staff from the Walpole Island Heritage Centre to maintain ecosystem diversity. This management strategy, which continues today, has resulted in some of the only populations of endangered or threatened plants and animals in the Great Lakes region. These include *Agalinis gattingeri* (Small) Small (round-stem false foxglove), *Agalinis skinneriana* (Alph. Wood) Britton (Skinner's false foxglove), *Gentiana alba* Muhl. ex Nutt. (plain gentian), *Solidago speciosa* Nutt. var. *rigidiuscula* Torr. & A. Gray (showy goldenrod), *Cypripedium candidum* Muhl. ex Willd. (white lady's slipper), *Polygala incarnata* L. (procession flower), *Gymnocladus dioicus* (L.) K. Koch (Kentucky coffee tree), *Aletris farinosa* L. (white colicroot), *Hydrastis canadensis* L.(goldenseal), *Liatris spicata* (L.) Willd. (dense blazing star), *Rosa setigera* Michx. (climbing rose), *Symphyotrichum praealtum* (Poir.) G.L. Nesom (willowleaf aster), *Ptelea trifoliata* L. (common hop tree), *Hibiscus moscheutos* Cav. (crimson-eyed rose mallow), *Quercus shumardii* (Palmer) Stoynoff & Hess (Shumard's oak), *Platanthera leucophaea* (Nutt.) Lindl. (prairie white-fringed orchid), and *Oligoneuron riddellii* (Frank ex Riddell) Rydb. (Riddell's goldenrod). Among these endangered or threatened plants, one crop wild relative that stands out is *Gymnocladus dioicus* (Kentucky coffee tree) a pod-bearing tree that was used as a food and coffee substitute by the Meskwaki, Pawnee, and Winnebago (Moerman 2017).

5.4 Designing Sustainable Collaborations

Working with tribal communities is more about building and sustaining relationships than narrowly focusing on project end goals and short-term deliverables. *How can plant researchers resolve institutional and agency expectations and goals with those of the indigenous communities they seek to work with?* First the Memorandum for the Heads of Executive Departments and Agencies and the Independent Regulatory Agencies clarifying Presidential Executive Order 13175 (President Bill Clinton) given by President Barack Obama on November 5, 2009 (Obama 2009) must be carefully read and understood. In these documents, guidelines for productive collaborations are stated, including "meaningful and timely form of consultation concerning the development, administration, and enforcement of regulations that have tribal implications." Planning documents and executive summaries are available for each agency that clarify how to achieve the goals of this Executive Order (Kenney 2012). Unfortunately, researchers are often not fully educated about the policies and guidelines they are supposed to follow, while the tribal communities they wish to work with are knowledgeable about these guidelines. Greater competency can be achieved if those presenting proposals to a tribal government frame it within the guidelines outlined in Executive Order 13175 to respect and honor tribal self-governance and sovereignty. To better illuminate how successful collaborations can be created between plant researchers and tribal communities, three case studies will be explored.

5.4.1 Case Study 1: Collaboration with the Walpole Island First Nation

In this case study, I worked with the people of the Walpole Island First Nation, located in present-day southeastern Ontario. This tribal First Nation exerts sovereign control on the plant material within their borders. For example, in 2002 my project to work with the citizens, business, and Bkejwanong Natural Heritage Program of the Walpole Island community on an ethnobotany project in the Great Lakes region was approved (Herron 2002). However, because both the common and rare, including endangered, flora had previously been extensively collected by researchers at the University of Michigan, my request to collect plant voucher specimens was not granted. Instead, I photographed plants and pasted photos onto a set of voucher specimen herbarium sheets—a very unconventional archival practice.

To work effectively with native communities, there needs to be a collaborative effort that equally includes the opinions of a particular community in the research. Collaboration is important because of the colonial history of removing the agency of tribes resulting in a general distrustfulness of community outsiders. My graduate work included an approved collection of heritage white flour corn by purchasing it from a tribal vendor. By respecting the community's wishes, this laid a foundation for trust and respect that is still intact 15 years later. In 2017, my initial efforts to engage in true collaboration were acknowledged when the University of Michigan convened a group of tribal communities, which included the Walpole Island First Nation and plant researchers, including myself to work on a maize restoration project (LaPorte 2017). Some of the Walpole Island First Nation tribal staff who were active participants in my graduate research project from 1999 to 2001 have expressed an interest in this new collaborative restoration project on maize. This heritage corn,

Fig. 5.1 (**a**, **b**) Collaboration with Walpole Island First Nation at University of Michigan Heritage Seeds Workshop in May 2017. (**a**) Tribal elder David White from Walpole Island First Nation and Scott Herron holding the white flour corn heritage seeds that Scott collected in 2001 during a workshop 16 years later. (**b**) Close-up image of the tribal heirloom variety of white flour corn (above) from 2001 along with another much older white flour corn (below) grown out by the University of Michigan in 1935 by Volney H. Jones

which is no longer grown by the Walpole Island First Nation, is the focus of a legal memorandum of understanding to bring the seeds out of a museum collection and grow them at the Matthaei Botanical Gardens, located in Ann Arbor, Michigan (Fig. 5.1). If successful, most of the seeds would be gifted back to the tribe, with a few fresh seeds returned to the museum collection (LaPorte 2017).

5.4.2 Case Study 2: Native Plant Restoration in Michigan's Upper Peninsula

Michigan's Upper Peninsula is a landmass separated from the Lower Peninsula by a five-mile stretch of the Great Lakes known as the Straits of Mackinac. This landmass is a home to five federally recognized Native American sovereign tribes (Inter-Tribal Council of Michigan 2017). Beginning in 2009, the Cedar Tree Institute, a nonprofit organization based out of Marquette, Michigan, has operated as the organizational and fiscal managers of an intertribal and US Forest Service project known as the Zaagkii Wings and Seeds Project (http://wingsandseeds.org/). During the previous 8 years of the project, 12 botanical workshops on native plants and pollinator restoration were conducted. Each of the five tribes in Michigan's Upper Peninsula hosted one or more of the workshops. One workshop was designed to inventory the crop wild relatives of cranberries growing on the Keweenaw Bay Indian Community's (KBIC) reservation land along Lake Superior. This workshop was led by both US Forest Service and USDA Agricultural Research Services staff and hosted by KBIC staff in their tribal facilities. It led to the collection of both voucher specimens and genetic material (leaf and fruit tissue) from both *Vaccinium macrocarpon* Aiton (large cranberry) and *V. oxycoccos* L. (small cranberry) (USDA Botany in the News 2014). According to US Forest Service Deputy Undersecretary Butch Blazer, this project could serve as a model for collaborative co-management of natural resources (Schultz 2015). He recommended that the Zaagkii Project continue and expand its efforts to assist tribes with technology transfer relating to climate challenges, native plants, and pollinators (Schultz 2015).

5.4.3 Case Study 3: Wildrice Eco-cultural Restoration in the Great Lakes

Ten years of collaboration (2006–2017) between myself and Roger LaBine, a Lac Vieux Desert Band of Lake Superior Chippewa tribal member and wildrice expert, has provided reflections on best management and planning efforts related to *Zizania palustris* L. (Robinson et al. 2009). Numerous multiday workshops known as *wildrice camps* have instructed hundreds of people from diverse backgrounds on the tools necessary for harvesting, processing, and cooking wildrice (Herron and LaBine 2016). Often these wildrice camps involved a restoration component where a portion of the hand-harvested grain is reseeded into specific waterbodies of interest by the tribal and agency partners (Fig. 5.2).

For some tribes in the Great Lakes, their natural resources staff are fully focused on fisheries and wildlife, as these activities can be major economic and subsistence drivers of these rural communities (Mattes 2017). Only select tribes including the Keweenaw Bay Indian Community located along the south shore of Lake Superior have natural resource staff dedicated to native plants including wildrice. However, in Michigan, wildrice is not a protected plant. Thus, tribal conservation plans often include reseeding new lakes and waterbodies that seem suitable to wildrice. The State of Michigan has prioritized aquatic invasive plant reduction in lakes including Houghton Lake, the state's largest inland lake once home to vast populations of wildrice. There has been a decline in wildrice since the Eurasian water milfoil (*Myriophyllum spicatum* L.) has required intensive herbicide treatment over many years (Bonnette 1998; Ustipak 1995). Restoration attempts in 2012 were not successful due to continued herbicide treatment of this lake. Only a recent change in the environmental engineering consulting firm managing Houghton Lake gives hope that the lake is moving from a treatment phase to a restoration phase, including that of wildrice. The US Forest Service and US National Parks Service have sought to include wildrice restoration in their plans for managed waterbodies (Jackson 2016;

Fig. 5.2 (**a–d**) Wildrice in various stages of processing at wildrice camps in Alberta, Michigan, in 2017. (**a**) Wildrice being weighed before sealing and distribution to rice camp participants. (**b**) Hand harvest and small batch processed wildrice that is finished and ready for distribution/consumption. (**c**) Fresh wildrice being parched over an open fire using cedar wooden paddle carved by rice camp participant. (**d**) Participants from a Keweenaw Bay Indian Community youth wildrice camp in August 2017 at Michigan Technological University's Ford Camp

Schoewe 2017). Working with Roger LaBine, who grew up within the wildrice culture and has taken the role as head rice instructor across Michigan and beyond, has led to some changes in the way both teaching and restoration efforts are carried forth. Back in the late twentieth century, agency staff tossed handfuls or bucketfuls of freshly harvested wildrice into a lake, river, or pond. Over the past decade, we have developed clear guidelines for how to eco-culturally restore wildrice into new locations. The spirit of wildrice is taught, including its role in the migration stories of the Ojibwe and other Anishinaabek tribal nations and its role as the caloric base of the aquatic food chain. Also communicated is the necessity to regularly visit and honor the place, the plants, and the spirit of new and existing wildrice communities (Herron and LaBine 2016).

5.5 Concluding Thoughts

North America has a highly diversified landscape with regional and local priorities driven by the local stakeholders. The above described case studies took place in the Great Lakes ecoregion of the United States. While some of the experiences are transferable across place and time, others are products of the cultures and conservation priorities of the particular tribal and colonial settler governments and citizens. Overall these case studies demonstrate a strong cross-cultural understanding and respect. Past inequities and the former management and conservation strategies driven by the dominant culture that lacked understanding of the roles indigenous practices had in the current landscape and species distribution patterns must be acknowledged. This acknowledgment will facilitate the future of co-management to benefit all humans and the ecosystems that we live in. There must be respect for both the known and unknown. The indigenous philosophy is to both not fear the unknown or to cast off the past but instead to learn from the past so that our grandchildren's children can inherit the earth from us in a healthy, even if changed, state, is the way to move forward.

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Part II
Conservation Strategies

Chapter 6
The Gene Pool Concept Applied to Crop Wild Relatives: An Evolutionary Perspective

Richard E. Miller and Colin K. Khoury

Abstract Crop wild relatives (CWR) can provide important resources for the genetic improvement of cultivated species. Because crops are often related to many wild species and because exploration of CWR for useful traits can take many years and substantial resources, the categorization of CWR based on a comprehensive assessment of their potential for use is an important knowledge foundation for breeding programs. The initial approach for categorizing CWR was based on crossing studies to empirically establish which species were interfertile with the crop. The foundational concept of distinct gene pools published almost 50 years ago was developed from these observations. However, the task of experimentally assessing all potential CWR proved too vast; therefore, proxies based on phylogenetic and other advanced scientific information have been explored. A current major approach to categorize CWR aims to comprehensively synthesize experimental data, taxonomic information, and phylogenetic studies. This approach very often ends up relying not only on the synthesis of data but also intuition and expert opinion and is therefore difficult to apply widely in a reproducible manner. Here, we explore the potential for a stronger standardization of the categorization method, with focus on evolutionary relationships among species combined with information on patterns of interfertility between species. Evolutionary relationships can be revealed with increasing resolution via next-generation sequencing, through the application of the multispecies coalescent model and using focused analyses on species discovery and delimitation that bridge population genetics and phylogenetics fields. Evolutionary studies of reproductive isolation can inform the understanding of patterns of interfertility in plants. For CWR, prezygotic postpollination reproductive barriers and intrinsic postzygotic barriers are the most important factors and determine the probability

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R. E. Miller (✉)
Flower Diversity Research Institute, Hammond, LA, USA

C. K. Khoury
USDA, Agricultural Research Service, Center for Agricultural Resources Research,
National Laboratory for Genetic Resources Preservation, Fort Collins, CO, USA

International Center for Tropical Agriculture (CIAT), Cali, Colombia
e-mail: colin.khoury@ars.usda.gov; c.khoury@cgiar.org

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of producing viable and fertile offspring. To further the assessment of CWR for use in plant breeding, we present observed and predicted gene pool indices. The observed index quantifies patterns of interfertility based on fertilization success, seed production, offspring viability, and hybrid fertility. The predicted gene pool index requires further development of the understanding of quantitative and qualitative relationships between reproductive barriers, measures of genetic relatedness, and other relevant characteristics for crops and their wild relatives.

Keywords Multispecies coalescent model · Postzygotic reproductive barriers · Reproductive isolation · Solanaceae · Species delimitation · Targeted gene capture · Unified species concept

6.1 Introduction

Everyone interested in biological diversity has a sense of what a species is. We recognize collections of individual organisms that look similar and give them common names, distinguishing these individuals from other dissimilar groups. For example, the bald eagle (*Haliaeetus leucocephalus* L.) is a North American sea eagle (sea eagles: Haliaeetinae) with a white head and tail and a brown body; it is a very distinctive large raptor within its range. It is easy to recognize as a different species from the larger golden eagle (*Aquila chrysaetos* L.) (booted eagles: Aquilinae) (Lerner and Mindell 2005).

A familiar definition of a species is the biological species concept, developed by the ornithologist and evolutionary biologist, Ernst Mayr (1942). The concept states "species are groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups." The focus of this influential definition (discussed by de Queiroz 2005) is whether populations interbreed or not, or in a more abstract sense, whether there is gene flow between diverging lineages (Petit and Excoffier 2009). This definition can certainly be applied to bald eagles and golden eagles. Even though these birds of prey are sympatric in some areas, they do not interbreed. It is interesting that the biological species concept makes no mention of whether species are morphologically distinct, even though naturalists' identification of species are most often based on visible characteristics (Coyne and Orr 1998).

Among flowering plants, it is common to observe groups of closely related species that are difficult to distinguish. These confusing plants also may hybridize and produce intermediate forms in the wild (Stebbins 1950; Grant 1981). Many species closely related to crops appear to belong to hybridizing complexes (Small 1984; Ellstrand et al. 1999; Rieseberg et al. 2007). This ability to produce viable offspring from interspecific hybrids can be advantageous to plant breeders. Owing to the interfertility between crops and their crop wild relatives (CWR), the genetic variation among these resources can be harnessed to introduce traits into crops to meet challenges, including pathogens, stressful environments, and changes in management practices (Hajjar and Hodgkin 2007).

Even species that are phenotypically distinct and reproductively isolated from crops may be considered CWR. Research has demonstrated that for many plant species, barriers acting early in the speciation process contribute most to reproductive isolation among distinct species (Martin and Willis 2007; Lowry et al. 2008). Artificial crosses between crops and their wild relatives can circumvent these early-acting barriers, leading to the successful production of viable hybrid offspring.

Crops are domesticated plant species (Brozynska et al. 2016) or sometimes complex combinations of more than one species, especially if they are of polyploid origin (e.g., bread wheat (*Triticum aestivum* L.) [International Wheat Genome Sequencing Consortium 2014]). An important goal in plant breeding is to identify the crop gene pool (Vincent et al. 2013), which includes the cultivated forms of the crop species, the wild species of the crop, and the crop's progenitors (Brozynska et al. 2016). Also included within the crop gene pool are closely related species, even if they did not serve as direct progenitors.

For some crops, there may be dozens or even hundreds of related congeneric species, for example, in the genus *Solanum* L. (Vorontsova et al. 2013; Hardigan et al. 2015). It is therefore critical to be able to discern which species are likely to be most successful in plant breeding efforts. To accomplish this, both a foundational knowledge of the evolutionary relationships among crops and their wild relatives and a comprehensive understanding of the relationship between patterns of interfertility and genetic relatedness and reproductive characteristics of CWR are needed.

6.2 A History of the Gene Pool Concept for CWR

Crop wild relatives are not merely the relatives of crops. All angiosperms share a common ancestor; therefore, all angiosperms are related. The practical implication of being defined as a CWR is that a plant species has some potential to be useful in a breeding program (Harlan and de Wet 1971). There is no taxonomic or evolutionary entity that corresponds to the notion of CWR; rather it is a concept specific to crop science and plant breeding.

The two main uses of the gene pool concept are to delineate the taxa that are CWR and then to identify levels of interfertility between these taxa and the associated crop. The early and very influential system for recognizing gene pools was developed by Harlan and de Wet (1971). They proposed three tiers of gene pools. The first tier corresponded to the biological species including the crop and, therefore by definition, individuals that exhibited no barriers to reproduction. The second tier included other taxa that could cross with the crop but with difficulty. Harlan and de Wet (1971) alluded to what would now be considered linkage drag, the retention of undesirable traits in the crop-wild relative hybrid (Prohens et al. 2017), making the use of these materials feasible but problematic. Finally, they recognized a tertiary group of taxa that were at the limits of interfertility, which could produce a few anomalous seeds or could be utilized only via radical techniques such as embryo rescue (Jansky 2006). Harlan and de Wet (1971) struggled to reconcile the biological diversity included within cultivated plants and their relatives with classic taxonomic categories, especially at the subspecific level, realizing that equivalent gene pool categories were unlikely.

The data required to make the assessment of placing CWR into Harlan and de Wet's gene pool tiers come from crossing studies to establish levels of interfertility. These are very challenging experiments to carry out and require substantial effort. A recent study of reproductive barriers among species of *Jaltomata* Schltdl. (Solanaceae) (Kostyun and Moyle 2017) provides an excellent example of the magnitude of what is needed to determine levels of interfertility among species. The authors investigated 10 species and performed crosses in a full diallel with typically 6 or 7 individual pairs for each interspecific cross and 8–10 crosses per pair, resulting in 4481 crosses. The study included additional control crosses (the number of which depended on the breeding system of the species involved—self-compatible versus self-incompatible).

These crossing experiments require a large greenhouse space and a significant investment in time. Furthermore, such controlled cross-pollinations entail careful manipulations of the flowers, such as emasculations of the dams, which is tedious (Eserman 2012). Given the tremendous effort needed to determine levels of interfertility among groups of species (Bohs 1991; Smith and Baum 2007; Eserman 2012; Plazas et al. 2016), it is thus not surprising that the comprehensive experiments required to confidently assign species to different tiers of the gene pool are often lacking for CWR, particularly for those related to non-major staple crops.

Proposals have been forwarded to use alternative sources of information to assign CWR to gene pools. One system is to use the existing taxonomies for species to assign CWR to gene pools, with the assumption that taxonomic classification in part reflects levels of genetic relatedness (Maxted et al. 2006). The taxon groups proposed by Maxted et al. (2006) included five taxa: the crop and its wild conspecifics, followed by members of the same series or section, subgenus, genus, and finally by different genera in the same tribe. The benefit of using the taxon group for designating CWR to different tiers is that more species can be considered with this scheme without requiring extensive experimental data. Unfortunately, this is a very blunt tool for assigning CWR to categories that are meant to reflect levels of interfertility. For example, generic status should reflect taxon group 4. Considering two solanaceous genera, *Solanum* L. with approximately 1500 species (Weese and Bohs 2007) and *Iochroma* Benth. with 24 species (Smith and Baum 2007), it seems highly unlikely that all of the species within each genus represent the same level of phylogenetic relatedness to the other members.

One reason genera are not a good proxy for levels of interfertility among species is that taxonomic categories above the species level are arbitrary and therefore often reflect the history of the treatment of the group rather than genetic relatedness. For example, within the tribe that represents morning glories with spiny pollen, the Ipomoeeae Hall. f., the Old World treatment of morning glories by Verdcourt (1963) retained earlier circumscribed genera within the tribe (e.g., *Argyreia* Lour., *Stictocardia* Hallier f.), while in the New World treatment of the morning glories, Austin (1997) dissolved previously recognized genera within the broad and cumbersome genus *Ipomoea* L. (e.g., *Batatas* Choisy, which contains sweetpotato, and *Pharbitis* Choisy). Put another way, levels of interfertility are not uniform for a particular taxonomic level. For example, among 11 close relatives of sweetpotato (members of *Ipomoea* series *Batatas* (Choisy) D.F. Austin), levels of interfertility ranged from very high to extremely low (Diaz et al. 1996), whereas among different genera within the Iochrominae, Smith and Baum (2007) reported high levels of interfertility throughout the tribe. Another important observation is that rates of speciation are not uniform across lineages, with some genera capturing high levels of diversity of recent rapid radiations (Kay et al. 2005). Thus, the taxon group proposal, while making an important effort to overcome the limitation set by reliance on hybridization evaluations, does not provide an accurate indicator of interfertility that can be used confidently.

Wiersema and León (2016) recognized both the pragmatic challenges of the Harlan and De Wet (1971) gene pool concept and the limitations of a system emphasizing taxonomic hierarchies (Maxted et al. 2006). They proceeded to marshal all available information, including taxonomic investigations combined with phylogenetic analyses and genetic and breeding studies, to delineate CWR and place them in categories of genetic relatedness as an updated approximation of the Harlan and de Wet (1971) gene pools. With this system, Wiersema and León designated the CWR of 208 crops from within 109 genera, based on an exhaustive review of the literature (J. Wiersema, pers. comm.). In the subsequent sections, we outline some of the most important concepts, methods, and sources of data used to inform an updated gene pool concept starting with consideration of the fundamental unit of CWR, the species.

6.3 What Is a Species?

Characterizing the complex relationships commonly observed among CWR requires a more encompassing notion of a species than the biological species concept (Levin 1979; Luckow 1995; de Queiroz 2005). Fortunately, after decades of discussion, a unified species concept has been successfully developed (Hey 2006; De Queiroz 2007). An important foundation of this idea is the evolutionary species concept, wherein species are considered separately evolving metapopulation lineages, which are populations with ancestor-descendant relationships that share the same evolutionary trajectory.

The important challenge then is to take this unified conceptual view of an evolutionary species and develop an operational approach that allow taxa to be recognized and delineated. The unified species concept provided the needed synthesis bringing together various operational species definitions under a single umbrella (De Queiroz 2007). The insight of de Queiroz (2007) was that a suite of properties could be extracted from the many species definitions and integrated, recognizing speciation as an extended process.

Two populations of a single species can be envisioned to diverge over time into two lineages, finally resulting in two clearly separate species. The speciation process is an extended one, where a series of steps cause the daughter lineages to differ to an ever-greater degree (de Queiroz 2007). Between the pairs of lineages, reproductive barriers develop during the speciation process, but not in the same sequence for all species. Diverging lineages may develop different fertilization systems; become adapted to different niches; accumulate random mutations increasing genetic differences; develop diagnosable, fixed differences; become phenetically distinguishable; or other changes. Furthermore, a phylogenetic analysis would find the lineages to be reciprocally monophyletic. An examination of gene genealogies for the different lineages would detect some gene histories exhibiting exclusive coalescence of alleles (de Queiroz 2007). At what point along the speciation continuum a researcher defines speciation to have occurred may depend on their area of expertise. From a genetic modeling point of view, perhaps 5% gene flow may be a useful operational criterion (Wiens and Servedio 2000). From a phylogenetic perspective, monophyly might be emphasized (Donoghue 1985). However, a broad approach using all evidence is now considered to be the most informative, allowing the data to tell the speciation story without a preconceived notion of which properties are definitive (de Queiroz 2007; O'Meara 2009; Fujita et al. 2012; Carstens et al. 2013).

Traditional taxonomic research emphasizes morphological characteristics to assign populations to described species. The treatment of *Ipomoea* in Bolivia (Wood et al. 2015) provides an elegant example of a morphology-based taxonomy. Descriptions and dichotomous keys were provided for 102 Bolivian morning glories species, with distribution maps, line drawings, and photographs for select species. Eighteen novel species were introduced, including a new member of the *Batatas* group, the closest relatives of sweetpotato (*Ipomoea batatas* (L.) Lam.).

But, when morphological analyses are combined with genetic data, as well as experimental crosses, a more complicated picture of relationships can emerge. The CWR of sweetpotato are considered members of the *Batatas* group, a hybridizing complex of 16 species distributed from Central United States to northern Argentina (Khoury et al. 2015). In an investigation that represents a snapshot of this complex, 154 Carolina populations representing four species (*Ipomoea cordatotriloba* Dennst., *I. lacunosa* L., *I. leucantha* Jacq., and an undescribed species referred to as *I. austinii* [non *I. austinii* Infante-Bet.]) revealed that *I. cordatotriloba* and *I. austinii* were morphologically distinct from the other taxa, while *I. lacunosa* and *I. leucantha* were morphologically similar, although with white and purple corollas, respectively (Duncan and Rausher 2013a, b). In contrast, *I. cordatotriloba* and *I. lacunosa*, while morphologically distinct, were not genetically differentiated and only exhibited partial reproductive isolation, whereas *I. austinii* and *I. leucantha* were genetically distinct. These complicated patterns of evolutionary relationships are consistent with the expectations of the early stages of the expanded speciation process. A more definitive species delineation for the CWR of sweetpotato thus requires further work to integrate traditional and newer tools to better discern the identities of taxa, with a view of their status within the speciation process.

CWR of other crops would be similarly expected to exhibit characteristics of taxa early in speciation. Among the collective populations and species represented by such groups, there may be population structure related to geographic barriers to gene flow, species diagnosed based on subtle morphological features, gene flow that may be ongoing between diverging lineages, and introgressive hybridization that may be occurring. These complications may all occur simultaneously among a group of CWR.

Thankfully, the potential to untangle complex evolutionary relationships and determine species boundaries is ever higher, due to the updated synthesis definition of a species, including the useful conceptual framework for the speciation process. In addition, excellent sources of genetic data and a rapidly developing toolkit of analytical methods are at researchers' disposal. A brief description of the most important recent technical, theoretical, and analytical advances is provided below.

6.4 Genetic Data for Understanding Evolutionary Relationships Among CWR

Genetic analysis techniques, specifically next-generation sequencing (NGS), can provide a wealth of DNA sequence data useful for examining evolutionary relationships (McCormack et al. 2013; Soltis et al. 2013). These methods have made it feasible, with a concerted effort and great expertise, to sequence entire nuclear genomes (Albert et al. 2013). For example, the complex and massive hexaploid bread wheat genome was recently sequenced (International Wheat Genome Sequencing Consortium 2014). It was particularly challenging because the polyploid AABBDD structure of the genome meant that three pairs of each gene exist. Furthermore, the wheat genome is riddled with repeats from transposable elements, making genome assembly extremely complicated. The near-complete sequence of the wheat genome was developed through a combination of high coverage with short reads from Illumina sequences and long reads from Pac Bio sequences. Approximately 100,000 CPU hours of computer time were required to stitch together the pieces to assemble 15,343,750,409 base pairs into a single genome (Zimin et al. 2017).

Whole nuclear genome sequences, especially for crops, are being completed at an amazing pace; at least 30 crop species have been sequenced (Morrell et al. 2012; Brozynska et al. 2016). The rice genome is notable because of its simplicity and has been a model for gene identification. In addition, although it is not a crop, the vast information for the model organism *Arabidopsis thaliana* (L.) Heynh. provides the foundation for the understanding of the molecular genetics of plants (The Arabidopsis Genome Initiative 2000).

Given these advances, quickly and inexpensively obtaining nuclear genome sequences of CWR species to determine evolutionary relationships may someday be possible, although this is not presently feasible (Soltis et al. 2013). Luckily, various methods are being developed that provide valuable information based on a reduced genome sample (Mascher et al. 2013; McCormack et al. 2013; Hirsch et al. 2014). These more limited samples can be obtained by genome skimming, restriction site-associated DNA sequencing, and targeted enrichment/gene capture. The strengths and weaknesses of these methods have been reviewed elsewhere (Hirsch et al. 2014; Heyduk et al. 2016).

It is valuable to consider the gene capture approach in detail, as this technique may be most informative at population and species levels, making it particularly suitable for untangling the complex relationships among CWR (McCormack et al. 2013; Lemmon and Lemmon 2013; Weitemier et al. 2014; Grover et al. 2015). The foundation of this method for estimating evolutionary relationships is to obtain sequences for sets of orthologous genes for the taxa under consideration. This is a major challenge because many genes are members of large and complex gene families (Morrell et al. 2012).

A set of probes (e.g., RNA baits) is designed to target the orthologous genes and to selectively sequence portions of the genome. Ideally, a reference nuclear genome is available among the taxa sampled or for a closely related species. Transcriptome sequences also can be used to develop the probe set, and for this the resources of the OneKP transcriptome project (http://www.onekp.com) are invaluable. At the simplest level, probes can be designed with a collection of expressed sequence tags (ESTs), as was successfully done in a study of the phylogenetics of the Asteraceae (Mandel et al. 2014).

After designing a set of probes, these RNA baits are hybridized to the DNA of the taxa included in the study. The hybridized samples are withdrawn from the pool of probes and DNA and are sequenced using a high-throughput sequencer (Illumina). The reads from the selective sequencing are meant to include only orthologous loci, but postsequencing filtering is needed. For example, the reads can be mapped onto the exon reference sequences to confirm that only orthologs are included in the analyses. The aligned sequences from these efforts can then be included in a wide array of population genetic and phylogenetic analyses.

The phylogenetic investigation of the palm genus *Sabal* Adans. by Heyduk et al. (2015) provides a good example of the elements of a targeted enrichment research project. This study examined relationships among a recalcitrant group of 15 species with interesting biogeography. To apply the gene capture approach, a set of 120-bp RNA baits was developed, benefiting from the availability of nuclear genome sequences, specifically from the date palm, *Phoenix dactylifera* L., and the African oil palm, *Elaeis guineensis* Jacq. (Heyduk et al. 2015). In addition, information from three transcriptomes assemblies (*Cocos nucifera* L., *Nypa fruticans* Wurmb., and *Sabal bermudana* L.H. Bailey) were available from the OneKP project (www.onekp.com). Together, this information was used to identify 837 exon sequences from 176 nuclear genes. The baits were hybridized with DNA from all 15 *Sabal* species, as well as two outgroup taxa. The sequences captured (both exon and intron) ranged from 48,965 to 355,729 base pairs. On average 159 of the 176 targeted nuclear genes were obtained among the taxa. The multiple-gene dataset was then analyzed using three coalescent-based phylogenetic methods (STAR [Liu et al. 2009]; MP-EST [Liu et al. 2010]; *BEAST 1.7.5 [Heled and Drummond 2010]). A well-resolved species tree was recovered that was largely congruent among the different coalescent-based methods. The biogeography of these palms also was resolved, identifying a Central and South American clade, a United States clade, and a clade of mostly Caribbean species. However, recent relationships remained ambiguous even with the large multigene dataset. Population-level sampling will be needed to understand the cause of this poor resolution.

It would be ideal to have a set of probes available to generate multigene datasets of orthologous loci that could be used for all CWR. But the magnitude of this endeavor is daunting, given the number of species of interest. For example, Vincent et al. (2013) and Dempewolf et al. (2014) estimated there may be thousands of potential CWR species. Animal studies have made use of an almost universal set of ultra-conserved elements (UCEs) that can be employed across a wide sample of faunal diversity (Faircloth et al. 2012). An effort is underway to identify a universal set of orthologous conserved loci in flowering plants (Buddenhagen et al. 2016). However, whole-genome duplication events are common across angiosperms, creating complicated genomes and important genetic differences among lineages (Duarte et al. 2010; Albert et al. 2013; Weitemier et al. 2014). A more feasible alternative is to develop a probe set for each plant order (Soltis et al. 2013). Nuclear plant genomes are already available for 30 crop species that represent 13 plant orders and as such could provide a foundation. However, a question that remains is how far-reaching a single set of probes can be. Comer et al. (2016) demonstrated that the probe set Heyduk et al. (2015) designed for the palm genus *Sabal* could be applied more broadly to explore phylogenetic relationships among exemplars of the palm subfamily Arecoideae. However, could RNA baits specifically designed for the CWR of potato (*Solanum tuberosum* L.), for example, be equally useful for resolving fine-scale relationships among the CWR of other members of the Solanaceae, such as tomato (*Solanum lycopersicum* L.), eggplant (*Solanum melongena* L.), chili pepper (*Capsicum annuum* L.), and tobacco (*Nicotiana tabacum* L.)? The answer to this question awaits further research.

6.5 Theoretical Foundation for Understanding Evolutionary Relationships Among CWR

The coalescent model is a stochastic process that can be used to explore relationships among populations, as well as among closely related species, making it excellent for understanding the evolution of CWR. DNA sequence polymorphism data are used to evaluate a wide range of population genetic processes simulated under the coalescent model. A scenario of particular relevance to CWR is when two populations diverge in isolation and where there is no gene flow between them. Population-level divergence of the coalescent process is therefore extended to provide a simple model of speciation or the multispecies coalescent. This model can be used to estimate evolutionary relationships, as well as accommodate other complicated processes, such as migration.

Taking a step back, an important model of genes evolving in populations is the Wright-Fisher model of genetic drift (Wakeley 2009). In the simplest case, the frequencies of two alleles at a single locus change from one generation to the next (for a haploid organism, without overlapping generations). Lineages are followed forward through time, and they branch when an individual randomly produces two or more offspring; lineages end when individuals do not reproduce. The result is random changes in gene frequencies from one generation to the next. In the absence of natural selection and mutation, owing to finite population size and stochasticity, one allele will eventually become fixed, while the other is lost. The dynamics of polymorphic loci in this hypothetical population provide a null model that can be used to examine how other evolutionary processes may modify patterns of polymorphisms.

The coalescent model is a significant extension of the Wright-Fisher model (Degnan and Rosenberg 2009). This stochastic model of gene evolution incorporates the insight of considering the dynamics of gene histories going backwards in time. In coalescent theory, genetic polymorphisms are the result of genealogical and mutational histories (Nordborg 2001; Rosenberg and Nordborg 2002). Lineages coalesce when individuals are produced by the same parent. For a population of individuals, ancestry is traced back through time, with the number of lineages decreasing, until a single individual is reached, representing the most recent common ancestor (MRCA) (Nordborg 2001). This pattern of gene genealogy provides the foundation for the coalescent approach, with neutral allelic variants mapped onto the genealogy, providing a separate description of the pattern of mutation.

The coalescent theory shares the same assumptions of the Wright-Fisher model (discrete generations, constant effective population size within populations, no population structure, no selection) (Rosenberg and Nordborg 2002; Degnan and Rosenberg 2009). This approach provides a springboard for examining how other processes affect the genealogy, such as population subdivision, species migration, hybridization, horizontal gene transfer between species, recombination, changes in population size, and geographic structure (Nordborg 2001; Degnan and Rosenberg 2009). More specifically, simulations model how the genealogy may vary because of different processes, without concern with the random process of mutation. However, the pattern of mutation among individuals carries the empirical information that allows the underlying unobserved genealogy to be compared to the simulated scenarios (Nordborg 2001).

For the study of crops and their wild relatives, the primary interest is an expanded version of the coalescent approach—the multispecies coalescent (Liu et al. 2015). In this model, the pattern of mutations and gene genealogies is maintained, and the species tree is added. For example, consider two descendant populations that split and no longer exchange genes with each other. In essence, there are two independent coalescent processes in the diverging lineages. In this scenario, divergence time can be estimated, realizing that all coalescent events must occur in the ancestor. This model of divergence without gene flow can be considered a null model of the splitting of species, providing the bridge between coalescent theory and phylogenetics (Wakeley 2009, 2013).

A fundamental realization with the coalescent approach is that the gene genealogies, or gene trees, are not the same as species trees (Edwards 2009). It is now well established that equating a gene tree with a species tree can lead to misleading inferences (Degnan and Rosenberg 2009). The focus of the coalescent approach is to understand the dynamics of multiple gene trees, nested within the context of a species tree. The signal from these gene trees provides the basis for an exploration of the full range of dynamics among populations and species while paying attention to the processes that give rise to gene tree discordance (incomplete lineage sorting, horizontal gene transfer, hybridization, natural selection, gene duplication) (Degnan and Rosenberg 2009).

6.6 Species Delimitation of CWR

Two major goals of systematics are to discover and describe species and then determine the phylogenetic relationships among taxa (Wiens 2007; O'Meara 2009). Species discovery using genetic data requires multiple population samples for each taxon. Carstens et al. (2013) provide an excellent review of available methods for species discovery (e.g., Structurama, Gaussian clustering, general mixed Yule coalescent model, O'Meara's heuristic method). An important point of their discussion is that each approach has assumptions that may be violated in some way by the real situation of the study system. Therefore, a suite of analyses should be explored, with a final assessment of species boundaries made from a careful synthesis of the results. It is useful to remember that not all diverging populations are expected to clearly exhibit the properties that demarcate separate species.

For the purposes of this discussion, we will consider STRUCTURE, a Bayesian model-based algorithm that is one of the more popular methods of species discovery (Pritchard et al. 2000; Hubisz et al. 2009). A STRUCTURE analysis for a diploid species employs polymorphic alleles at numerous unlinked loci. Samples of individuals are assigned to clusters based on allele frequencies under Hardy-Weinberg disequilibrium (Fujita et al. 2012; Carstens et al. 2013). The number of clusters (k) supported by the data is evaluated in a series of analyses with different numbers of k (Evanno et al. 2005). These clusters are an estimate of the number of lineages supported by the data, and then individuals are assigned to the lineages with varying levels of confidence.

STRUCTURE analysis can employ a model with or without admixture (Falush et al. 2003). Including admixture allows individuals to be assigned to multiple lineages and therefore provides an estimate of the level of gene flow occurring between them. If the lineages are interpreted to be separate species, this analysis provides an examination of the strength of species boundaries. A strong example of application of STRUCTURE for species delineation is the investigation of relationships among brinjal (*Solanum melongena*), scarlet (*S. aethiopicum* L.), and gboma (*S. macrocarpon* L.) eggplants and 14 species of their wild relatives (Acquadro et al. 2017). Acquadro et al. (2017) used next-generation sequencing to identify 75,399 polymorphic sites among 76 individuals. In their initial analysis of this large dataset, Acquadro et al. (2017) used fastSTRUCTURE (Raj et al. 2014) to identify four major subgroups, including one that included brinjal eggplant, its wild progenitor, and its close relatives (*S. melongena* with *S. insanum* L., *S. incanum* L., *S. linnaeanum* Hepper & P.-M.L. Jaeger, and *S. lichtensteinii* Willd.). However, individuals identified as *S. campylacanthum* Hochst. ex A. Rich., *S. lidii* Sunding, *S. tomentosum* L., *S. vespertilio* Aiton, and *S. violaceum* Ortega showed evidence of admixture, exhibiting membership in as many as three distinct lineages. Following the initial analysis for population genetic structure, closer examination of the subgroups was carried out with additional explorations, using fastSTRUCTURE to identify genetically based clusters within these subgroups.

Following species discovery, the next step is species validation. Again, Carstens et al. (2013) advocate applying multiple methods to the data (BPP, spedeSTEM). Bayesian phylogenetics and phylogeography (BPP) provides an example of this process (Yang and Rannala 2010; Rannala and Yang 2017). This is a Bayesian inference procedure for multilocus sequence data, based on the multispecies coalescent. The analysis typically begins with a guide tree, and then various scenarios varying in species number and relationships (topologies) are explored using a Markov chain Monte Carlo search. The maximum number of species is specified at the start, and then posterior probabilities for the nodes of the species tree are obtained, using the sequence data. In other words, BPP evaluates the strength of various possibilities of species relationships and number of species. The outcome of these analyses is identified lineages. Comparing BPP to other validation approaches suggests this method tends to oversplit genetic relationships (Carstens et al. 2013). Therefore, it is important to integrate the genetic results with information from biogeography, ecology, morphology, and, when possible, the degree of reproductive isolation to make a synthetic assessment of the number of species and their relationships. With careful examination of the number of species included in the population samples and their validated relationships, one can proceed with greater confidence to examine phylogenetic relationships among taxa. Various phylogenetic methods have been developed based on the multispecies coalescent model that can complete this final step (ASTRAL, Mirarab and Warnow 2015; *BEAST 1.7.5; Heled and Drummond, 2010; MP-EST, Liu et al. 2010; STAR, Liu et al. 2009).

An example of this integrative approach of combining a BPP analysis with morphological and ecogeographic data is the study of group of rare North American orchids, the *Corallorhiza striata* Lindl. complex (Barrett and Freudenstein 2011). In this taxonomically challenging group, relationships were examined among two species, *C. bentleyi* Freudenst. and the widespread *C. striata* Lindl. The results based on genetic data, morphology, and geography were not congruent. BPP resolved four lineages: *C. bentleyi* + *C. striata* var. *involuta* (Greenm.) Freudenst. and three distinct populations of *C. striata* from different regions in North America. In contrast, morphological and geographic evidence supported the separation of *C. bentleyi* and *C. striata* var. *involuta*. The authors settled on recognizing three species, *C. bentleyi*, *C. involuta*, and a widespread *C. striata* s.s. The orchid study not only demonstrates the application of BPP in combination with morphological and biogeographic analyses but also provides an example of the extended speciation process where different properties were found to delineate various combinations of taxa.

These analyses offer new options to resolve long-standing challenges with regard to CWR gene pools. Returning to the CWR of sweetpotato, the *Batatas* group represents a hybridizing complex, which has challenged taxonomic efforts (Austin 1978; Wood et al. 2015). The characteristics and status of the CWR of sweetpotato may be common for other groups of CWR (e.g., the following are crops and their wild relatives: alfalfa [*Medicago sativa* L.], barley [*Hordeum vulgare* L.], beans [*Phaseolus vulgaris* L.], cassava [*Manihot esculenta* Crantz], carrot [*Daucus carota* L.], cotton [*Gossypium hirsutum* L. and *G. barbadense* L.], eggplants [*Solanum melongena, S. aethiopicum, S. macrocarpon*], maize [*Zea mays* L. subsp. *mays*], potato [*Solanum tuberosum*], rice [*Oryza sativa* L. and *O. glaberrima* Steud.], sorghum [*Sorghum bicolor* (L.) Moench], soybean [*Glycine max* (L.) Merr.], sugarcane [*Saccharum officinarum* L.], sunflower [*Helianthus annuus* L.], and wheat [*Triticum aestivum*]) where species are diagnosed on disputable characteristics, complex patterns of reproduction have been detected, incomplete barriers to reproduction are present, and cryptic species have been discovered (Small 1984; Ellstrand et al. 1999; Rieseberg et al. 2007; Grover et al. 2015; Hardigan et al. 2015; Bredeson et al. 2016; Acquadro et al. 2017).

6.7 What Determines Patterns of Interfertility Among Plants?

An evolutionary perspective can inform the understanding of patterns of interfertility among CWR from an investigation of reproductive isolation in plants (Lowry et al. 2008; Baack et al. 2015). Determining patterns of reproductive isolation is at the foundation of understanding speciation. As discussed above, the framework of the extended speciation process portrays a view where along the speciation continuum different barriers develop until species are completely reproductively isolated from one another and thereby exhibit an increasing number of the properties that characterize separate species.

To better understand reproductive isolation, different stages of reproductive barriers have been recognized. One of the main distinctions is made between barriers that operate before or after zygote formation (Coyne and Orr 1998). Prezygotic isolating mechanisms involve such factors as differences in ecogeography or elaborate floral structures that result in adaptations to alternative pollinators that act to eliminate gene flow between species pairs before mating takes place (Stebbins 1950). In contrast, postzygotic isolating mechanisms involve factors such as hybrid inviability, where the zygote does not develop into a normal embryo (Stebbins 1950). For plants, a further distinction is made between prezygotic barriers that act prior to pollination (e.g., differences in flowering time) and prezygotic postpollination barriers (e.g., interactions between pollen and stigma) (Tiffin et al. 2001).

An important model for understanding the development of reproductive barriers is the Bateson-Muller-Dobzhansky model or Dobzhansky-Muller incompatibilities (DMIs) model, where allelic incompatibilities between diverging species increase, leading to hybrid inviability and sterility (Coyne and Orr 1998; Tiffin et al. 2001; Turelli and Moyle 2007). The model is based on mutations at two or more loci developing in two diverging populations. If the two populations interbreed after mutations have accumulated, some of the genetic combinations will be incompatible. One prediction from the DMI model is that the level of reproductive isolation between species pairs is positively associated with genetic distance, which is thought to reflect time since divergence. In other words, with greater divergence, reduced hybrid fitness is expected. To explore this prediction, Coyne and Orr (1989) used an extensive literature survey of 119 *Drosophila* species pairs where data were available for at least one measure of reproductive isolation and information was available to estimate genetic distances. From this survey, they found strong support for a positive correlation between reproductive isolation and genetic distance (Coyne and Orr 1989, 1997). This research initiative was extended to other animal groups, and the general trend continued to hold (Mendelson 2003). Moyle et al. (2004) scoured the literature to apply the Coyne and Orr (1989) approach to published studies for plants and found three genera with suitable data. For one group, *Silene* L., they found a pattern of increasing reproductive isolation with greater genetic distances, consistent with the animal studies (Moyle et al. 2004). However, for the other two genera (*Glycine* Willd. and *Streptanthus* Nutt.), the pattern was not as straightforward (Moyle et al. 2004). They concluded that other factors may have contributed to the pattern of interfertility, such as insufficient time to develop effective reproductive barriers (*Streptanthus*), unusual crossing relationships of individual species (*Glycine falcata* Benth.), or perhaps genes of large effect causing the patterns of reproductive isolation (*Glycine*).

When considering whether a pair of plant species is likely to be crossed successfully, intuitively one might predict that species very different in appearance will be less likely to produce viable hybrid offspring than similar-looking species. For example, *Jaltomata* species exhibit striking floral diversity. One might expect the two species with rotate corollas, *Jaltomata sinuosa* (Miers) Mione and *J. antillana* (Krug & Urb.) D'Arcy, to be interfertile and similarly for the pair with tubular corollas, *J. aijana* Mione & S. Leiva and *J. incahuasina* Mione & S. Leiva (Fig. 6.1). The notion is that traits related to floral divergence may have pleiotropic effects on development so that greater overall morphological divergence would indirectly lead to genetic incompatibilities and thereby result in reduced postmating prezygotic and intrinsic postzygotic reproductive isolation. Kostyun and Moyle (2017) tested this idea in an investigation of 10 species of *Jaltomata*. They did not find support for floral divergence being a strong predictor of the strength of reproductive isolation. In contrast, a significant correlation between genetic distance and intrinsic postzygotic reproductive isolation was detected, providing additional support for this general relationship.

Animal studies of reproductive isolation have shown that prezygotic isolating mechanisms are often stronger than postzygotic isolating mechanisms and evolve

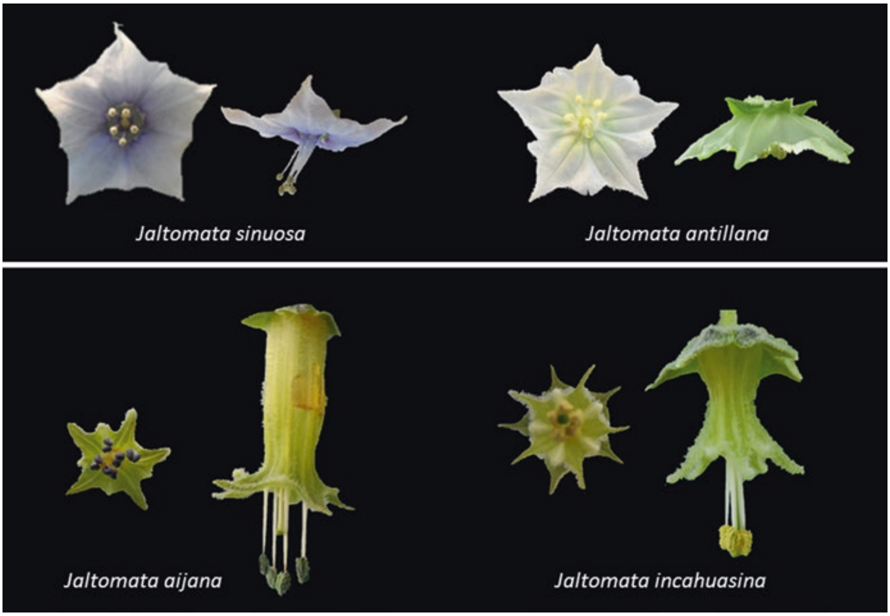

Fig. 6.1 Floral diversity among four *Jaltomata* species with two species exhibiting rotate corollas and two species with tubular corollas. (Photographs courtesy Jamie Kostyun)

sooner between diverging species pairs (Coyne and Orr 1989, 1997; Mendelson 2003). The results from investigations of suites of individual reproductive barriers between plant species are consistent with findings from animals, with prezygotic isolating mechanisms, both before and after pollination, providing a much greater contribution than postzygotic isolating mechanisms to overall reproductive isolation (Rieseberg and Willis 2007; Lowry et al. 2008). For CWR, it is informative to consider prezygotic isolating mechanisms, such as ecogeographic separation or flowering time differences, to help to explain the diversification of these species. However, for plant breeding purposes, artificial crosses circumvent these prepollination prezygotic barriers to reproduction. Therefore, postpollination prezygotic barriers and intrinsic postzygotic barriers should be the primary focus for the successful utilization of CWR.

If the barriers to reproduction are the result of an accumulation of nuclear genetic changes acting in a Mendelian fashion, then the patterns of reproductive isolation would be predicted to be symmetrical regardless of which species serves as the sire and which serves as the dam (Tiffin et al. 2001). However, asymmetry in patterns of reproductive isolation is common in plants based on which species acts as the pollen or seed parent (Tiffin et al. 2001; Lowry et al. 2008; Plazas et al. 2016; Kostyun and Moyle 2017). In a survey of 14 genera, Tiffin et al. (2001) detected significant asymmetries in three life history stages: seed production, hybrid viability, and hybrid sterility. In another review, Lowry et al. (2008) found asymmetries were greatest for postmating barriers, notably hybrid seed set and viability. These asymmetries indicate factors that are likely to contribute to reproductive isolation, including gametophyte-sporophyte interactions (Pease et al. 2016), problems with endosperm development because of genomic incompatibilities causing defective seeds (Lafon-Placette and Köhler 2016), cytonuclear interactions that may lead to hybrid male sterility (Chen et al. 2017), and others (reviewed in Turelli and Moyle 2007).

An interesting example of a gametophyte-sporophyte interaction comes from an investigation of tomato (*Solanum lycopersicum*) and its relatives. In a study involving the wild species *Solanum pennellii* Correll, Pease et al. (2016) identified the molecular genetic basis of postmating prezygotic reproductive isolation. They examined the fascinating system of unilateral incompatibility, where *S. pennellii* (self-incompatible) pollen tubes can extend into *S. lycopersicum* (self-compatible) styles to fertilize the ovaries, whereas *S. lycopersicum* pollen grains are prematurely blocked in the styles of *S. pennellii*. Using an elegant experimental design, transcriptomes were obtained from a combination of tissues that allowed contrasting gene expression patterns to be used to identify candidate genes potentially responsible for the observed reproductive barriers (Pease et al. 2016).

6.8 Further Innovating the Gene Pool Concept for CWR

The focus on genetic relatedness of the Wiersema and León (2016) proposal is excellent and represents the best effort to date to use an understanding of evolutionary relationships to categorize CWR across many crop gene pools. However, with many thousands of potentially useful species to evaluate (Vincent et al. 2013; Dempewolf et al. 2014), a more widely applicable and standardized system of assessment would be extremely helpful, building on a quantitative understanding of the relationship between genetic relatedness and patterns of interfertility among CWR. Three components stand out as required to accomplish the creation of this system. The first is to determine to the greatest extent possible evolutionary relationships among CWR, as has been discussed. The remaining components build on the understanding of the evolution of reproductive isolation in plants by developing two quantitative measures of the patterns of interfertility among CWR: observed and predicted gene pool indices.

The observed gene pool index can be constructed based on results from crossing studies involving CWR and crops. It is similar to estimating the level of reproductive isolation for studies of speciation by carefully examining all potential reproductive barriers and calculating their cumulative effect (Martin and Willis 2007; Lowry et al. 2008). However, the observed gene pool index places a primary focus on reproductive processes that reduce the level of interfertility related to fertilization success, seed viability, offspring vigor, and hybrid fertility. Separate values are needed based on which species is the female or male parent for each cross due to the aforementioned asymmetries in the action of reproductive barriers. The observed gene pool index should in sum reflect the probability of producing viable and fertile hybrid offspring from a select cross.

More challenging is transforming the observed gene pool index into a predicted gene pool index. As in the majority of cases crossing studies have not been comprehensively performed for all species related to all crops, the predictive index ends up in actuality being the foundation for developing an independent and reproducible assessment system.

The first step to develop the predicted gene pool index borrows from the approach of Coyne and Orr (1989, 1997) and Moyle et al. (2004) to use available results from the literature to survey relationships between genetic relatedness and patterns of interfertility among CWR and their crop species. Specifically, reproductive barriers related to postpollination prezygotic barriers and intrinsic postzygotic reproductive barriers are of interest. The objectives of the survey are to determine if a broad relationship exists between the observed gene pool index and measures of genetic relatedness (e.g., based on nucleotide substitutions, patristic distances, etc.). However, equally important is to bring attention to those cases that deviate from the general pattern and to consider various factors that may reduce interfertility in these cases, including gametophyte-sporophyte interactions disrupting fertilization, problems with endosperm development leading to inviable seeds, and cytonuclear interactions resulting in hybrid male sterility. There may be a quantitative relationship between interfertility and genetic distance, but an additional qualitative pattern may emerge owing to failed pollination, seeds dying, and hybrids being sterile. Therefore, in addition to obtaining quantitative estimates of genetic relatedness, it is important to identify characteristics of the CWR that may also determine patterns of interfertility, such as ploidy level, mating system, pollen size and style length, properties of the endosperm, and characteristics of the mitochondrial genome.

It may be necessary to carry out careful experimental crossing studies coupled with phylogenetic investigations for groups of CWR where a knowledge gap exists. In some cases, the number of populations of CWR to consider may be too numerous to be included in the labor-intensive efforts of crossing studies; therefore, a careful exemplar sample should reflect the important characteristics that may determine the pattern of interfertility. Further analyses providing results for various gene pools, and then testing the results across other plant groups, will be necessary to determine whether particular interfertility factors are highly important across most or all groups. With this knowledge, further studies on other groups can be more efficiently targeted.

A study of eggplant and its wild relatives (Plazas et al. 2016) provides an indication of the methods needed to develop an observed gene pool index. Crosses were carried out between 6 accessions of eggplant (*Solanum melongena*) and 19 accessions of 12 wild species, sampling from the three tiers of the gene pool (Plazas et al. 2016). Fruit set, number of seed per fruit, and germination success were recorded with *S. melongena* as both the female and male parent. Fruit set was as high as 48%, maximum seeds per fruit was 2.7, and in one case 92% germination was observed. From these values, the number of viable seeds produced from 100 crosses can be estimated. For example, the crosses between *S. melongena* and its progenitor *S. insanum* would result in 44 viable offspring (17.8% fruit set, 2.67 seeds/fruit, 92.2% germination) with eggplant as the female parent and 55 viable offspring (33.3% fruit set, 2.18 seeds/fruit, 75.8% germination) with eggplant as the male parent.

To develop a corollary predictive gene pool index for eggplant and its wild relatives, genetic data obtained by Acquadro et al. (2017) and the phylogenetic investigations by Levin et al. (2006), Vorontsova et al. (2013), and Aubriot et al. (2016) can provide the foundation. The taxa sampled by Vorontsova et al. (2013) included many species in the Plazas et al. (2016) study. Based on results from two nuclear genes and a chloroplast marker, Vorontsova et al. (2013) resolved clades that can be used as an estimate of patristic distance. The phylogeny indicates that *Solanum melongena* was nested with its putative progenitor, *S. insanum*, with *S. incanum* as the sister species to this clade. *Solanum linnaeanum* and *S. lichtensteinii* were the next closest relatives, whereas *S. violaceum*, *S. dasyphyllum* Schumach. & Thonn., *S. anguivi* Lam., *S. tomentosum*, *S. pyracanthos* Lam., *S. elaeagnifolium* Cav., and *S. torvum* Sw. were increasingly distantly related and were not part of the eggplant clade.

These phylogenetic relationships can be compared with the patterns of interfertility from the Plazas et al. (2016) crossing study, focusing on fruit set with the most complete data (percent fruit set for hybrids with eggplant as the female or male parent, respectively). A general pattern emerges where species more closely related to *S. melongena* had intermediate levels of fruit set: *S. insanum* (18, 33), *S. incanum* (18, 25), *S. linnaeanum* (9, 48), *S. lichtensteinii* (17, 18), *S. violaceum* (5, 0) *S. dasyphyllum* (24, 11), *S. anguivi* (15, 34), and *S. tomentosum* (12, 32). In contrast, more distantly related species had low levels of fruit set: *S. pyracanthos* (0, 5), *S. elaeagnifolium* (0, 0), and *S. torvum* (3, 0). It is intriguing to note that the species that deviated from the general pattern, *S. violaceum*, was a taxon that exhibited admixture and showed membership in lineages characteristic of three other species (Acquadro et al. 2017). Among the species more closely related to eggplant, no linear trend between genetic relatedness and fruit set was detected. This suggests that factors acting in a qualitative fashion are more likely to be determining the pattern of interfertility among eggplant and these CWR.

There is a vast literature on the systematics of crops and their wild relatives, and, in addition, there are decades of crossing studies involving CWR—many more than could be considered here. The greatest gap at this point is the application of a quantitative approach to make a strong connection between evolutionary relationships and patterns of interfertility among CWR. Using published research to explore this relationship, looking for general trends, predictable patterns, and unusual special cases, is sure to provide insight and some surprises. Such a survey will help to identify gaps in knowledge that will hopefully inspire targeted further research. In combination, these investigations hold great promise to contribute to the development of the foundation understanding needed to anticipate with much greater accuracy how efficiently CWR can contribute to crop improvement.

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Chapter 7
Conservation Status and Threat Assessments for North American Crop Wild Relatives

Anne L. Frances, Adam B. Smith, and Colin K. Khoury

Abstract Conservation status and threat assessments evaluate species' relative risks of extinction globally, regionally, nationally, or locally and estimate the degree to which populations of species are already safeguarded in existing conservation systems, with the aim of exposing the critical gaps in current conservation. Results of the assessments can therefore aid in directing limited conservation resources to the species and populations that are most at-risk. This chapter introduces the roles of conservation status and threat assessments in informing conservation priorities for crop wild relatives in North America and provides an overview of the current results for US taxa. Methods to assess the conservation status and to perform threat assessments for North American crop wild relatives are well developed via NatureServe and the International Union for Conservation of Nature (IUCN) Red List, and the essential infrastructure to perform these analyses is present, at least in Canada and the US. Current conservation assessments for North American wild relatives need updating but already reveal a landscape of multiple complex threats

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A. L. Frances (✉)
NatureServe, Arlington, VA, USA
e-mail: anne_frances@natureserve.org

A. B. Smith
Missouri Botanical Garden, St. Louis, MO, USA
e-mail: adam.smith@mobot.org

C. K. Khoury
USDA, Agricultural Research Service, Center for Agricultural Resources Research, National Laboratory for Genetic Resources Preservation, Fort Collins, CO, USA

International Center for Tropical Agriculture (CIAT), Cali, Colombia
e-mail: colin.khoury@ars.usda.gov; c.khoury@cgiar.org

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and major gaps in the ex situ and in situ conservation of prioritized species. Further resources and concerted efforts are needed to update conservation assessments and then to use the results to inform efforts to fill the critical gaps in conservation.

Keywords Global rank · Red List · Ex situ · In situ · Threat assessment · Gap analysis · Conservation status

7.1 Introduction

The need to conserve crop wild relatives has long been recognized (Harlan 1976; Meilleur and Hodgkin 2004). Historically, most conservation actions have focused on ex situ, or off-site, collections, with emphasis on availability to researchers for crop breeding and other uses. In the last few decades, there has been an increased interest in in situ or on-site conservation of crop wild relatives (Convention on Biological Diversity 2011; Dempewolf et al. 2014). Complementary conservation, also called integrated plant conservation, includes both ex situ and in situ measures (Kramer et al. 2011). An integrated approach is generally seen as more effective than either individual method in conserving crop wild relatives because it enables naturally occurring populations to be subjected to continued natural selection, while also securely safeguarding genetic resource diversity and making it available to the research community (USDA Forest Service and Agricultural Research Service 2014; Moray et al. 2014; Fielder et al. 2015).

Integrated conservation applied to the full spectrum of crop wild relatives thus represents the ideal, but insufficient resources for conservation, competing priorities for the use of wildlands, and, perhaps most importantly, lack of awareness by decision-makers of the importance of crop wild relatives make actualization of this goal challenging. The current reality is that conservationists must choose their priorities, focusing their efforts on species and populations that are particularly threatened or have special cultural or genetic resource value. Robust information on the identities, distributions, threats, and realized or potential value of crop wild relatives provides the knowledge base needed to prioritize among these species.

This chapter introduces the roles of conservation status and threat assessments in informing conservation priorities for crop wild relatives in North America and provides an overview of the current results for US taxa. These assessments evaluate species' relative risks of extinction globally, regionally, nationally, or locally and estimate the degree to which populations of species are already safeguarded in existing conservation systems, with the aim of exposing the critical gaps in current conservation (Master 1991; Collen et al. 2016). Results of the assessments can therefore aid in directing limited conservation resources to the species and populations that are most at-risk.

7.2 Assessing the Conservation Status of Species

Most conservation efforts rely on information from status assessments to prioritize their work. Due to the recognized importance of these assessments, they are included in several international policy initiatives and strategies. For example, Target 2 of the Convention on Biological Diversity's Global Strategy for Plant Conservation calls for "an assessment of the conservation status of all known plant species, as far as possible, to guide conservation action" by 2020 (Convention on Biological Diversity 2012). Similarly, the North American Botanic Garden Strategy for Plant Conservation calls on botanic gardens to review and contribute to conservation status assessments of plants using criteria and standards developed by NatureServe and the International Union for Conservation of Nature (IUCN) (BGCI 2016). Finally, Target 12 of the Aichi Biodiversity Targets of the Convention on Biological Diversity's Strategic Plan recommends using the IUCN Red List of Threatened Species to identify imperiled species, prevent their extinction, and improve their conservation status, by 2020 (Convention on Biological Diversity 2011).

The two most widely used platforms for assessing the conservation status of species in North America are NatureServe Conservation Status Assessments and the IUCN Red List. The next sections provide an overview of each platform and a comparison between them.

7.2.1 NatureServe Conservation Status Assessments

The NatureServe Network in North America comprises over 65 independent programs representing subnational jurisdictions in Canada, the US, and Mexico, which collaborate in performing conservation status assessments and providing the results (called ranks) on a shared platform. The network gathers, analyzes, and distributes biodiversity data on species and ecosystems via an independent methodology from those used by the IUCN Red List and other pertinent conservation status assessments.

NatureServe ranks indicate the potential extinction or extirpation risk of taxa by systematically analyzing rarity, threats, and trends (Faber-Langendoen et al. 2012; Master et al. 2012). Ranks have been produced at least once for over 70,000 North American plant and animal taxa, including nearly every vascular plant occurring in Canada and the US. These results have been used extensively by US and Canadian state and federal agencies, including state natural heritage programs.

Ranks are completed at three nested, geographic scales: global (G), national (N), and subnational (S) (i.e., state and provincial). Data from subnational ranks are used to inform national and global ranks. Specifically, state natural heritage programs and Canadian data centers provide subnational-level data on species including mapped populations (element occurrences) and local threats and conditions. The use of common standards and methodology enables these data to be aggregated into national and global datasets that serve as the basis for national and global ranks. By indicating species imperilment at different jurisdictional scales, governments and decision-makers are better able to allocate resources for the most imperiled taxa in their respective jurisdictions while at the same time considering species' overall risks of extinction throughout their ranges (Faber-Langendoen et al. 2012).

Within each geographic scale, species and infraspecific taxa (i.e., varieties and subspecies) are ranked from most to least imperiled on a scale of 1–5 (Table 7.1). NatureServe ranks also include GX (presumed extinct) and GH (possibly extinct). Uncertainty in a global rank is expressed through range ranks, variant ranks, and

Table 7.1 NatureServe Conservation Status Ranks adapted from Master et al. (2012)

Global (G) rank	Definition
GX	Presumed extinct—species not located despite intensive searches and virtually no likelihood of rediscovery
GH	Possibly extinct—known from only historical occurrences but still some hope of rediscovery. There is evidence that the species may be extinct, but not enough to state this with certainty
G1	Critically imperiled—at very high risk of extinction due to extreme rarity (often five or fewer populations), very steep declines, or other factors
G2	Imperiled—at high risk of extinction or elimination due to very restricted range, very few populations, steep declines, or other factors
G3	Vulnerable—at moderate risk of extinction or elimination due to a restricted range, relatively few populations, recent and widespread declines, or other factors
G4	Apparently secure—uncommon but not rare; some cause for long-term concern due to declines or other factors
G5	Secure—common; widespread and abundant
Variant global ranks	
G#G#	Range rank—a numeric range rank (e.g., G2G3, G1G3) used to indicate uncertainty about the exact status of a taxon
GU	Unrankable—currently unrankable due to lack of information or due to substantially conflicting information about status or trends
GNR	Unranked—global rank not yet assessed
GNA	Not applicable—a conservation status rank is not applicable because the species is not a suitable target for conservation activities
Rank qualifiers	
?	Inexact numeric rank—denotes inexact numeric rank; this should not be used with any of the variant global conservation status ranks or GX or GH
Q	Questionable taxonomy that may reduce conservation priority—distinctiveness of this entity as a taxon at the current level is questionable; resolution of this uncertainty may result in change from a species to a subspecies or hybrid, or inclusion of this taxon or type in another taxon or type, with the resulting taxon having a lower-priority (numerically higher) conservation
C	Captive or cultivated only—at present presumed or possibly extinct in the wild across the entire native range but extant in cultivation, in captivity, as a naturalized population outside their native range or as a reintroduced population, not yet established. Possible ranks are GXC or GHC

rank qualifiers (Table 7.1). For example, taxa with questionable taxonomy that may affect the conservation assessment are assigned the rank qualifier of "Q."

Assessing the conservation status of a species requires detailed knowledge of its identity, distribution, population trends, and threats. NatureServe's ranking process uses eight core rank factors organized into three categories: rarity, threats, and trends (Master et al. 2012) (Table 7.2). Two additional factors are considered conditional and are used only when information on certain core factors is not available (Table 7.2; see Faber-Langendoen et al. 2012 for details). Using the rank calculator tool, factors are scaled and weighted consistently to score the contribution of each factor to extinction risk. The combined scores result in a calculated rank, which is reviewed by an expert who then assigns the final conservation status rank.

The three factor categories—rarity, threats, and trends—require a minimum amount of information for each species to calculate a conservation status assessment.

Table 7.2 Summary of NatureServe Conservation Status Rank Factors adapted from Master et al. (2012)

Factor category	Subcategory	Factor	Definition
Rarity	Range/distribution	Range extent	Minimum area that encompasses all present occurrences
		Area of occupancy	Area within the range extent occupied by a species
	Abundance/condition	Population size	Estimated total mature individuals occurring in wild populations within a species' natural range
		Number of occurrences	Number of discrete areas occupied by a species (e.g., subpopulations, populations, metapopulations)
		Number of occurrences with good viability	Number of occurrences with excellent-to-good viability, such that there is the likelihood of persistence under current conditions
		Environmental specificity[a]	Degree to which the species depends on a relatively scarce set of habitats, substrates, food types, or other factors within the overall range
Threats		Overall threat impact	Degree to which a species' viability is affected by extrinsic factors (stressors), characterized by scope and severity
		Intrinsic vulnerability[a]	Degree to which a species' inherent characteristics, such as life history, make it susceptible or resilient to stress
Trends		Long-term trend	Degree of past directional change in population, range extent, area of occupancy, or number of occurrences over the long term (ca. 200 years)
		Short-term trend	Degree of past directional change in population, range extent, area of occupancy, or number of occurrences in the short term, defined as within 10 years or three generations, whichever is longer

[a] Indicates conditional factors used only if information on certain core factors is not available

Rarity, which is weighed more heavily than threats and trends in NatureServe's ranks, includes five core factors and one conditional factor. Three of these factors (range extent/extent of occurrence, area of occupancy, and population size) are equivalent to Red List definitions. Threats are usually assessed by assigning an overall threat impact, although a species' intrinsic vulnerability may be used as a conditional factor when information on threats is not available. Threats are categorized using the hierarchy first published by Salafsky et al. (2008), while the threat impact score is calculated considering the scope, severity, and timing of present and future threats. The trend factors describe the degree of change in a species' range, distribution, abundance, or condition over the short term (within 10 years or three generations) or long term (ca. 200 years). Values for trends include estimates of increases, declines, and relative stability (Faber-Langendoen et al. 2012). Detailed guidance on the methodology, factors, and rank calculator is available on NatureServe's website (natureserve.org) and in Faber-Langendoen et al. (2012) and Master et al. (2012). Conservation status ranks of species and ecosystems are found on the NatureServe Explorer website (explorer.natureserve.org).

7.2.2 The IUCN Red List of Threatened Species

The IUCN is a global network focused on environmental conservation with over 1300 governmental and nongovernmental member organizations and supported by over 10,000 experts. The IUCN network has been instrumental in producing global environmental protection agreements such as the Convention on Biological Diversity and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

The IUCN Red List of Threatened Species (Red List) was established in 1964 with the goal of providing a baseline from which to measure and monitor the state of the world's biodiversity (Westwood et al. 2017). Like NatureServe's ranks, the Red List is designed to evaluate the relative risk of extinction among species with the purpose of highlighting species that are threatened or are facing a high risk of extinction.

Most Red List Assessments are completed by members of IUCN Species Survival Commission Specialist Groups and Red List Authorities, although anyone can request to assess or review a species. Specialist Groups and Red List Authorities are usually comprised of experts of taxonomic groups or geographic regions. For example, crop wild relatives in North America may be assessed by the Crop Wild Relative Specialist Group, the Red List Authority for North American Plants, or the Hawaiian Plant Specialist Group, to name a few (for a full list of plant specialist groups, see https://www.iucn.org/ssc-groups/plants-fungi). The Red List includes global-level assessments, although regional or national assessments may also be included for species endemic to single countries.

The IUCN Red List uses five quantitative criteria in a rule-based approach to determine if a species is threatened, near threatened, or least concern:

A. Declining population (past, present, and/or projected)
B. Geographic range size and fragmentation, decline, or fluctuations
C. Small population size and fragmentation, decline, or fluctuations
D. Very small population or very restricted distribution
E. Quantitative analysis of extinction risk (e.g., population viability analysis)

Threatened species include the categories of critically endangered, endangered, or vulnerable (Table 7.3). Many of the criteria also require the use of sub-criteria to further justify listing species as threatened or near threatened. The Red List Categories and Criteria (IUCN 2012) provides the methodology for assigning each of the criteria to a species, while detailed instructions and case studies are found in the guidelines (IUCN Standards and Petitions Subcommittee 2017). The IUCN provides a number of additional key documents, as well as all published Red List Assessments, on the Red List website (iucnredlist.org).

7.2.3 Comparison Between NatureServe and IUCN Red List Conservation Assessments

NatureServe and the IUCN Red List use many of the same concepts (such as the area of occupancy, extent of occurrence, and population size), underlying information, and methods for classifying and coding to inform status assessments (Salafsky

Table 7.3 IUCN Red List global status categories

Red List category	Definition
EX	Extinct—no reasonable doubt that the last individual has died
EW	Extinct in the wild—known only to survive in cultivation, in captivity or as a naturalized population (or populations) well outside the past range
CR	Critically endangered—facing an extremely high risk of extinction in the wild based on meeting any of the criteria A to E for critically endangered using the best available evidence
EN	Endangered—facing a very high risk of extinction in the wild based on meeting any of the criteria A to E for endangered using the best available evidence
VU	Vulnerable—facing a high risk of extinction in the wild based on meeting any of the criteria A to E for vulnerable using the best available evidence
NT	Near threatened—close to qualifying for or is likely to qualify for a threatened category (critically endangered, endangered, or vulnerable) based on an evaluation against the criteria
LC	Least concern—widespread and abundant taxa that have been evaluated against the criteria and do not qualify for critically endangered, endangered, vulnerable, or near threatened
DD	Data deficient—inadequate information to make a direct, or indirect, assessment of its risk of extinction based on its distribution and/or population status
NE	Not evaluated—not yet been evaluated against the criteria

Table 7.4 Comparable categories between NatureServe global rank and IUCN Red List category

NatureServe global rank	IUCN Red List category
Presumed extinct (GX)	Extinct (EX)
Presumed extinct in the wild[a] (GXC)	Extinct in the wild (EW)
Possibly extinct (GH)	Critically endangered (CR) (possibly extinct)
Possibly extinct in the wild[a] (GHC)	Critically endangered (CR) (possibly extinct)
Critically imperiled (G1)	Critically endangered (CR)
Critically imperiled (G1)	Endangered (EN)
Imperiled (G2)	Vulnerable (VU)
Vulnerable (G3)	Near threatened (NT)
Apparently secure (G4)	Least concern (LC)
Secure (G5)	Least concern (LC)
Unrankable (GU)	Data deficient (DD)

Adapted from Master et al. (2012)

[a] Species ranked GXC and GHC are presumed or possibly extinct in the wild across their entire native range but are extant in cultivation, in captivity, as a naturalized population (or populations) outside its historical native range, or as a reintroduced population not yet established. The C modifier is only used with status ranks at a global level and not a national or subnational level. Similarly, IUCN's EW status is only used at a global level

et al. 2008). Moreover, many of the thresholds between the different categories are set at the same approximate level, so in the majority of cases, the NatureServe rankings and the Red List categories largely align (Table 7.4). Both systems are dynamic and are updated, ideally based on new threats or changing population trends.

This said, the processes of evaluating the data and assigning ranks and categories by the systems differ. NatureServe ranks follow a weight-of-evidence approach with minimum criteria, whereas the IUCN Red List is based on applying a set of rules to the given criteria (Westwood et al. 2017). Red List Assessments place a higher emphasis on trends, while NatureServe ranks prioritize rarity in assessing extinction risk. Both platforms utilize methods and establish guidelines for addressing and expressing uncertainty in the underlying data; however, these methods differ substantially. For example, NatureServe uses multiple range ranks (e.g., G2G3, G1G3) to indicate uncertainty about the exact status of a species, while Red List Assessments include a category of near threatened to indicate a species close to qualifying for a threatened category. Although many of the same concepts are utilized by both platforms, NatureServe rankings cannot automatically be transferred over to IUCN Red List categories and vice versa. For reviews (albeit now outdated) of the NatureServe platform compared to the IUCN Red List as well as the Endangered Species Act (Endangered Species Act of 1973, 16 U.S.C. Sec 1531), see Master et al. (2012) and Regan et al. (2005).

In the case of the US and Canada, nearly all plant species and infraspecific taxa have been ranked by NatureServe at least once (ca. 25,000 taxa). In contrast, there are only approximately 3100 completed Red List Assessments for plant taxa in the two countries. Although NatureServe's ranks provide a useful baseline for evaluating conservation status, many global ranks have not been reviewed in over 10 years. Some taxa, especially those ranked G4 or G5, have not been reviewed in over 20 years. Both NatureServe and the Red List are aware of these data gaps and are working collaboratively to update conservation status information for North American plants. In particular, the Red List initiated the Plants for People (P4P) project to assess the conservation status of 6000 species of crop wild relatives, medicinal plants, timber trees, and palms (IUCN 2017a). Currently, Red List Assessments are underway for hundreds of crop wild relatives in Mesoamerica (IUCN 2017b).

7.3 Indications of the Conservation Status and Threats to US Crop Wild Relatives

Although the US has for numerous decades been actively involved in various ways with the conservation of its crop wild relatives (see, e.g., USDA Forest Service 2016 and Seiler et al. 2017), a national inventory of these species was published only recently (Khoury et al. 2013). Such an inventory is a foundational step to conservation as it identifies species of interest and prioritizes them by their potential value for crop breeding and other research. Once species of interest are identified, their conservation status can be used as a further criterion to prioritize their conservation.

Conservation status assessments for 76% of taxa listed in the US national inventory have been recorded in NatureServe (Khoury et al. 2013). Of these, 8 (0.2%) taxa were assessed as known or presumed extinct in the wild, 115 (3.3%) as globally critically imperiled, 111 (3.2%) as imperiled, 337 (9.6%) vulnerable, 798 (22.7%) apparently secure, and 2143 (61%) globally secure.

Of the species in the inventory, the IUCN Red List assesses 16 taxa as extinct, endangered, or vulnerable (IUCN 2012). Sixty-two taxa are also listed as endangered under the US Endangered Species Act (Endangered Species Act of 1973, 16 U.S.C. Sec 1531), 10 taxa as threatened, and 11 taxa as candidates for listing. Among the taxonomic groups with the largest absolute number of threatened taxa are members of the family Fabaceae, particularly within the genera *Astragalus*, *Lotus*, *Lupinus*, and *Trifolium*.

7.3.1 Threat Assessment for Critically Imperiled and Imperiled US Crop Wild Relatives

Identifying the threats to rare and endangered species is critical for guiding conservation action (Murray et al. 2014). Both the NatureServe and IUCN Red List conservation status assessments identify threats using a hierarchical threats taxonomy, with the first level representing broad categories of threats and the second more

Table 7.5. First- and second-level threats in the Threats Classification Scheme currently in use by the IUCN Red List and NatureServe

First-level threat	Second-level threat
1 Residential and commercial development	1.1 Housing and urban areas; 1.2 commercial and industrial areas; 1.3 tourism and recreation areas
2 Agriculture and aquaculture	2.1 Annual and perennial non-timber crops; 2.2 wood and pulp plantations; 2.3 livestock farming and ranching; 2.4 marine and freshwater aquaculture
3 Energy production and mining	3.1 Oil and gas drilling; 3.2 mining and quarrying; 3.3 renewable energy
4 Transportation and service corridors	4.1 Roads and railroads; 4.2 utility and service lines; 4.3 shipping lanes; 4.4 flight paths
5 Biological resource use	5.1 Hunting and collecting terrestrial animals; 5.2 gathering terrestrial plants; 5.3 logging and wood harvesting; 5.4 fishing and harvesting aquatic resources
6 Human intrusions and disturbance	6.1 Recreational activities; 6.2 war, civil unrest, and military exercises; 6.3 work and other activities
7 Natural system modifications	7.1 Fire and fire suppression; 7.2 dams and water management/use; 7.3 other ecosystem modifications
8 Invasive and other problematic species, genes, and diseases	8.1 Invasive non-native/alien species/diseases; 8.2 problematic native species/diseases; 8.3 introduced genetic material; 8.4 problematic species/diseases of unknown origin; 8.5 viral/prion-induced diseases; 8.6 diseases of unknown cause
9 Pollution	9.1 Domestic and urban wastewater; 9.2 industrial and military effluents; 9.3 agricultural and forestry effluents; 9.4 garbage and solid waste; 9.5 airborne pollutants; 9.6 excess energy
10 Geological events	10.1 Volcanoes; 10.2 earthquakes/tsunamis; 10.3 avalanches/landslides
11 Climate change and severe weather	11.1 Habitat shifting and alteration; 11.2 droughts; 11.3 temperature extremes; 11.4 storms and flooding; 11.5 other impacts

Third level threats are defined in some cases and can be found online (http://www.iucnredlist.org/technical-documents/classification-schemes/threats-classification-scheme). Threats are commonly specified using the hierarchical number plus full name

specific threats (Table 7.5). The threats hierarchy used by NatureServe and IUCN are based on the threats taxonomy published by Salafsky et al. (2008) but differ slightly from one another due to modifications to the taxonomy over time. Currently NatureServe has conducted formal assessments using the hierarchical threats taxonomy for 963 plant taxa distributed in the US, though Hernández-Yáñez et al. (2016) employed a systematic textual analysis to extend coverage to all 2733 US plant taxa that are critically imperiled (G1), imperiled (G2), possibly extinct (GH), possibly extinct in the wild (GHC), or listed or candidates for listing under the US Endangered Species Act. Here threat assessments for 214 US crop wild relatives are reported including 163 taxa analyzed by Hernández-Yáñez et al. (2016) plus an additional 51 taxa assessed for this chapter, using the same methods and standards.

Of the 214 taxa, 203 occur in the continental US and 11 in Hawaii. Only 22.4% (48 taxa) are listed under the US Endangered Species Act, with one additional species considered as a candidate. Seventy-nine percent of taxa (169) were documented as having at least one known threat, while 21 percent of taxa (45) had no documented threats. The distribution of threats was highly skewed, with most species having a few threats and a minority of species having either no threats or many threats.

First-level threats affected crop wild relatives differently than rare US plants as a whole. Across crop wild relatives, the most common threat was natural system modifications, affecting 44% of taxa, while across all US species, this threat was the fifth most common, affecting 29% of taxa (Hernández-Yáñez et al. 2016). Other common first-level threats to crop wild relatives included residential and commercial development (41% versus 31% for all US plant taxa); agriculture and aquaculture (32% versus 33%); invasive and other problematic species, pathogens, and genes (30% versus 43%); biological resource use (26% versus 15%); human intrusion and disturbance (20% versus 33%); and transportation and service corridors (19% versus 21%). Each of the other first-level threats affected fewer than 10% of crop wild relatives.

Second-level threats affecting >20% of US crop wild relatives included:

- Housing and urban areas (33% of species)
- Fire and fire suppression (28%)
- Invasive non-native/alien plants and animals (23%)
- Dams and water management/use (20%)

Threats from fire and fire suppression were largely due to the third-level threat, suppression in fire frequency/intensity (20%) and less to increase in fire frequency/intensity (4%) or unspecified changes in fire frequency/intensity (4%). The distribution of second-level threats across crop wild relatives suggests that many taxa are affected by direct land usurpation (e.g., urbanization and inundation by dams) as well as factors that alter competitive and consumer-resource dynamics (e.g., invasive species and fire suppression). Several significant threats also pertained to use of wild or cultivated plants, including:

- Wood and pulp plantations (17%)
- Logging and wood harvesting (16%)
- Annual and perennial non-timber crops (13%)
- Gathering terrestrial plants (13%)
- Livestock farming and ranching (10%)

Crop wild relatives were somewhat more likely to be affected by direct harvest (gathering terrestrial plants) than US taxa in general, though the overall rate of threat was low (13% of crop wild relatives versus 9% of all US taxa).

The distribution of second-level threats affecting wild relatives also differed substantially from those impacting all rare US taxa (Fig. 7.1). Crop wild relatives were more noticeably likely to be threatened by housing and urban areas, fire and fire

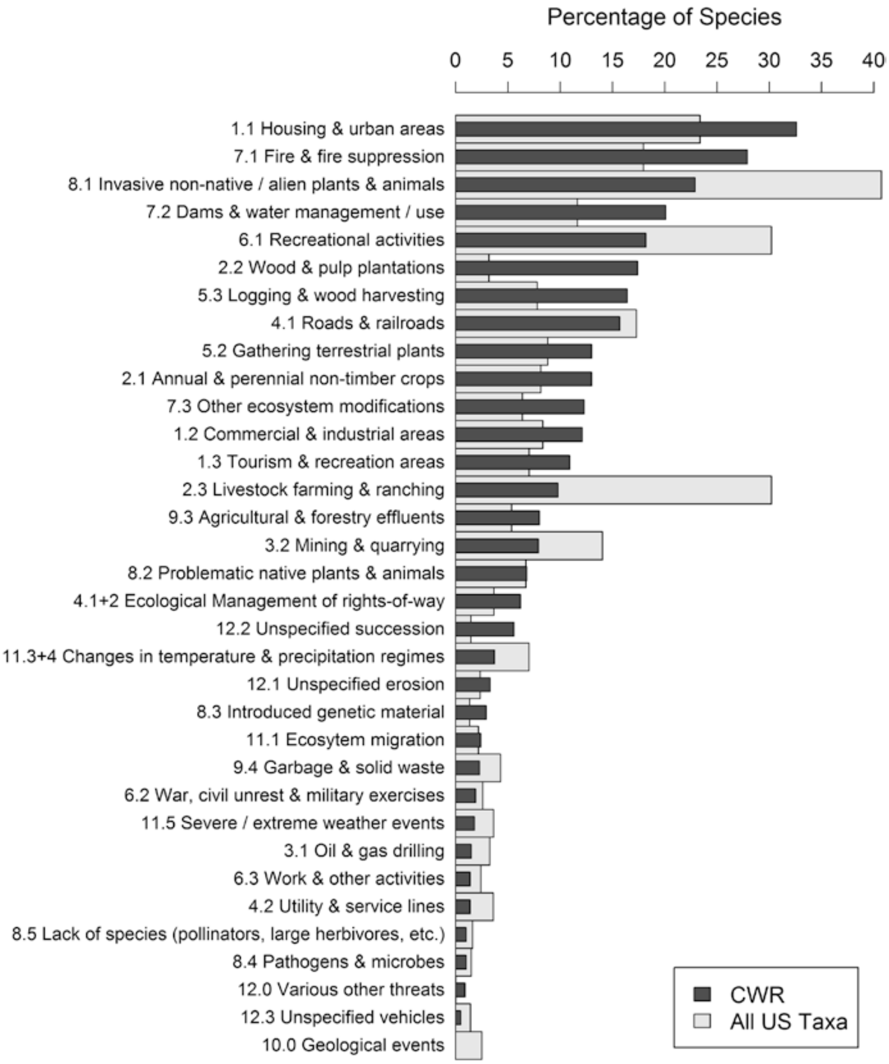

Fig. 7.1 Frequency of second-level threats affecting US crop wild relatives (CWR) (*n* = 214) and all rare US plants (*n* = 2733) (Hernández-Yáñez et al. 2016). Threats are listed in order from most common to least common among wild relatives. The frequency of most common threats affecting wild relatives is notably different from the frequency affecting rare plants in the US as a whole

suppression, dams and water management/use, and forestry operations, including wood and pulp plantations and logging and wood harvesting. In contrast, US taxa as a whole were more likely to be affected by invasive non-native/alien plants and animals, recreational activities, livestock farming and ranching, and mining and quarrying. Discrepancies between these two sets of taxa could arise due to differences in distribution across the US (e.g., Estill and Cruzen 2001), life form (e.g., Prescott and Stewart 2014), or innate sensitivity to anthropogenic activities (Murray et al. 2014).

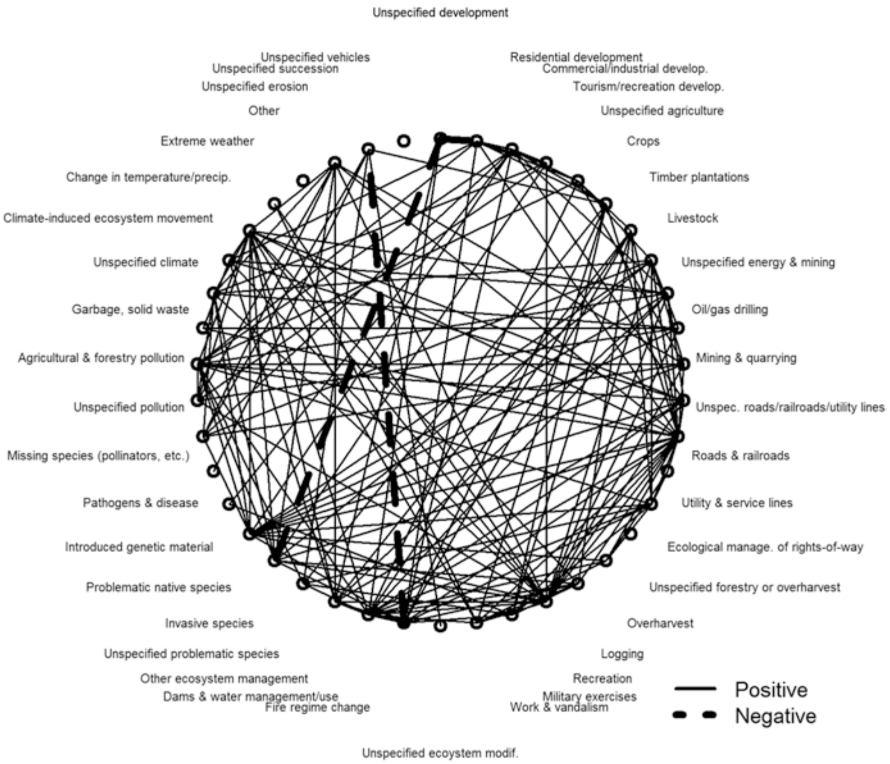

Fig. 7.2 Positive and negative pairwise associations between second-level threats affecting 214 rare US crop wild relatives. Nineteen percent of possible associations are significantly positive and <0.1% negative, whereas by chance only 5% of associations should be significant, with an equal split between positive and negative. The high rate of positive associations suggests threats acting in concert often affect wild relatives

Threats can co-occur to impart even more vulnerability than threats operating alone (Burgman et al. 2007; Budiharta et al. 2011; Jennings and Rohr 2011). Associations between threats can also offer opportunities for efficiencies in conservation efforts if they emanate from the same activity (e.g., agriculture is associated with land conversion, pollution from pesticides, introduction of invasive species, and dams and waterway diversions). Across crop wild relatives in the US, there were several positive associations between threats (Fig. 7.2). Of 861 possible pairwise associations between second-level threats, 19% (165) were significant and positive (χ_2 test on Yule's φ measure of association), although the rate of positive associations was even higher (47%) across all continental US rare taxa (Hernández-Yáñez et al. 2016). In contrast, <0.1% of associations between threats were significantly negative, a trend mirrored by continental US taxa as a whole. The co-occurrence of threats suggests that conservation actions for crop wild relatives must mitigate multiple and sometimes interacting impacts (Burgman et al. 2007; Budiharta et al. 2011).

7.3.2 Identifying Gaps in Conservation of US Crop Wild Relatives

Identifying current gaps in ex situ and in situ conservation of crop wild relatives is integral to determining the next steps needed to improve integrated conservation. Gap analysis methodologies are aimed at effectively identifying the populations and species most in need of further conservation action (Ramírez-Villegas et al. 2010; Castañeda-Álvarez et al. 2016).

7.3.2.1 Gap Analysis of Ex Situ Collections of Crop Wild Relatives

Gap analysis methods enabling estimates of the degree of representation of crop wild relatives (and other important plant genetic resources) in gene banks have progressed considerably since geographic information systems technologies began to be applied to conservation planning and as ecogeographic data has become more comprehensive (Hijmans et al. 2001; Hijmans and Spooner 2001; Olson et al. 2001; Jarvis et al. 2003; Hijmans et al. 2005; Phillips et al. 2006; Ramírez-Villegas et al. 2010; Parra-Quijano et al. 2012; Castañeda-Álvarez et al. 2016; Hengl et al. 2017; Fick and Hijmans 2017).

The basic steps in ex situ gap analysis for crop wild relatives include mapping or otherwise estimating the distributions of species and then comparing the original collecting localities of existing gene bank, botanic garden, and other collections against these distributions to both assess the representation of species ex situ and to expose the gaps in these collections. Gap analysis methods can also aid in locating which regions have the greatest richness in species, which can help to inform efficient collecting activities (Nabhan 1990).

Alongside basic assessments of taxonomic and geographic gaps in collections, such methods increasingly include environmental or ecological niche gaps (e.g., the degree of representation of the range of climates, soils, and habitats that species occupy) (Ramírez-Villegas et al. 2010; Parra-Quijano et al. 2012; Castañeda-Álvarez et al. 2016). Recent studies have additionally analyzed the representation of populations harboring potentially valuable agronomic traits, particularly with regard to abiotic stresses (Tapia et al. 2014; Khoury et al. 2015; Khoury et al. 2015a). As the generation of molecular information becomes increasingly cost-effective, such data are likely to be incorporated into gap analyses to more directly assess the current representation ex situ of useful genetic diversity (McCouch et al. 2013). More direct analyses of gaps in genetic diversity should help to mitigate some of the constraints inherent to methods relying on ecogeographic information as proxy for genetic diversity (e.g., Araújo and Guisan 2006; Hijmans and Graham 2006; Graham et al. 2008; Jimenez-Valverde et al. 2008; Loiselle et al. 2008; Costa et al. 2010; Lobo et al. 2010; Hijmans 2012; Gaiji et al. 2013), which may partly explain discrepancies in gap analysis prioritization results in comparison with expert opinion (Castañeda-Álvarez et al. 2016).

A comprehensive gap analysis for prioritized North American crop wild relatives has yet to be performed, although the process is underway in the US. A recent gap analysis of crop wild relatives in gene banks performed at the global level (Castañeda-Álvarez et al. 2016) produced worrying results. Of the 1100 wild species thought to be of greatest value worldwide to the improvement of food crops, almost 30% were completely missing from the world's gene banks, and over 70% were in urgent need of collecting. The US and Mexico were recognized among the most important hotspots, with many important native species inadequately represented ex situ. Gap analyses performed at the crop gene pool level and covering wild relatives native to North America have also revealed large gaps for most species (e.g., for apple [Volk et al. 2015], bean [Ramírez-Villegas et al. 2010], cotton [Wallace et al. 2009], potato [Castañeda-Álvarez et al. 2015], sweetpotato [Khoury et al. 2015a], and sunflower [Kantar et al. 2015]).

7.3.2.2 Gap Analysis of In Situ Conservation of Crop Wild Relatives

Similar to the state of production of ex situ gap analyses, comprehensive assessments of the level of protection of naturally occurring populations of North American crop wild relatives in designated protected areas have yet to be completed. What is clear is that while current federal, state, and other jurisdictional conservation policies in the region afford some protection for wild relatives, they clearly fall well short of providing adequate actively managed long-term in situ protection of the diversity of native wild genetic resource plants (see, e.g., Wilkes 2007). In only a handful of areas (i.e., the US Forest Service wild chile [*Capsicum annuum* var. *glabriusculum* (Dunal) Heiser and Pickersgill] preserve in Southern Arizona, US [USDA Forest Service 2016], and the Sierra de Manantlan Biosphere Reserve in Jalisco/Colima, Mexico, which conserves a wild relative of maize [*Zea diploperennis* H.H. Iltis, Doebley and R. Guzmán], various wild beans, and other crop wild relatives) does such conservation include active management plans with regularly scheduled monitoring of populations.

Two important administrative and legislative formats by which in situ conservation can be improved in the US are discussed below. Such federal and state, as well as other official threatened and endangered species prioritizations, provide critical justifications for conservation investment and should be strengthened to better protect North American wild relative species.

7.3.2.2.1 The Endangered Species Act

The Endangered Species Act (ESA) is a federal law in the US designed to protect imperiled species and the habitats upon which they depend. Endangered species under the ESA are in danger of extinction throughout all or a significant portion of their range, and threatened species are likely to become endangered within the foreseeable future. The US Fish and Wildlife Service and the National Marine Fisheries

Service determine which species are listed or are candidates for listing under the ESA. Threatened or endangered taxa under the ESA are eligible for federal protection, recovery planning, and funding for conservation actions.

While the ESA does confer protection to some threatened plants, neither the current number of listed plant species nor the funding allocated to their recovery is sufficient to ensure their protection. While 40 percent of vertebrates regarded by NatureServe as G1 (critically imperiled) or G2 (imperiled) are also listed under the US Endangered Species Act, only 20 percent of similarly ranked plants are federally listed (Evans et al. 2016). Federally listed plants receive less protection than federally listed animals. For example, even though nearly 60% of species on the US Endangered Species List are plants, they consistently receive less than 5% of state and federal funding (Negrón-Ortiz 2014). In addition, while federal agencies must consider the protection of listed ESA species in land planning projects, private landowners are only required to consider the protection of listed ESA animals and not plants.

7.3.2.2.2 State Wildlife Action Plans

State Wildlife Action Plans are important state-level processes in the US, which involve multi-year strategies to assess the health of wildlife and outline pathways to improved conservation. The plans aim to protect species before they become endangered and are custom-fitted to individual jurisdictional needs and priorities. State Wildlife Action Plans are consistently used to inform conservation actions at the state and national levels (Stein and Gravuer 2008).

Similar to the Endangered Species Act, plants are currently significantly underrepresented in State Wildlife Action Plans compared to animal species. States develop action plans to protect species designated of "Greatest Conservation Need". To date, only 15 of 56 US states and territories have included plants in their lists of Species of Greatest Conservation Need. One major challenge is that the national State Wildlife Grant Program continues to define wildlife as "fauna, and not flora," due to historical funding sources, including excise taxes on hunting equipment (1937 Pittman-Robertson Act) and fishing gear (1950 Dingell-Johnson Act) (Stein and Gravuer 2008). Such a definition precludes the use of program resources to work on plants, leaving jurisdictions to find alternative funding for flora.

7.4 Conclusion

Methods to assess the conservation status and to perform threat assessments for North American crop wild relatives are well developed via NatureServe and the IUCN Red List, and the essential infrastructure to perform these analyses is present, at least in Canada and the US. Current conservation assessments for North American wild relatives need updating but already reveal a landscape of multiple complex threats and major gaps in the ex situ and in situ conservation of prioritized species. Further resources and concerted efforts are needed to update conservation assessments and then to use the results to inform efforts to fill the critical gaps in conservation.

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Chapter 8
Sampling Wild Species to Conserve Genetic Diversity

Sean Hoban, Gayle Volk, Kanin J. Routson, Christina Walters, and Chris Richards

Abstract Sampling seed from natural populations of crop wild relatives requires choice of the locations and the amount of seed to sample. While this may seem like a simple choice, in fact careful planning of a collector's sampling strategy is needed to ensure that a crop wild collection will contain high genetic variation, which is in turn needed for high potential for breeding or selection. Here we first describe the different conservation targets and intensities at which a collector might sample. We then review research on the appropriate number of populations, plants, and seeds to collect, and we review different methodologies available for helping to make these decisions. We suggest that samplers reconsider the long-standing minimum of 50 samples per population, as this will be insufficient in some cases. We explain that the optimal minimum number of populations, samples, and seeds can be determined with modeling approaches (niche modeling, a genetic survey, or simulations) that use knowledge of a species' inherent traits (e.g., outcrossing rates) and geographic distribution. Lastly, we review some practical aspects of sampling, including the need to return to the source population to collect seed due to genetic change over time and the need to collect additional seed (sometimes 10 times as much seed) to account for loss of seed during storage and use. We also emphasize the utility of collecting abundant spatial and environmental data during seed sampling, as well as considering how samples will be used for accompanying genetic analyses, in order to make ex situ collections useful for research and breeding for many years to come.

S. Hoban (✉)
The Morton Arboretum, Center for Tree Science, Lisle, IL, USA
e-mail: shoban@mortonarb.org

G. Volk · C. Walters · C. Richards
USDA, Agricultural Research Service, Center for Agricultural Resources Research, National Laboratory for Genetic Resource Preservation, Fort Collins, CO, USA
e-mail: gayle.volk@ars.usda.gov; Christina.Walters@ars.usda.gov; Chris.Richards@ars.usda.gov

K. J. Routson
University of Arizona, Arid Lands Resource Sciences, Tucson, AZ, USA

Edge Mountain Research, Prescott, AZ, USA

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8.1 Introduction

Genetic diversity refers to the different variants that can exist for each gene and how those variants are distributed among chromosomes, individuals, populations, and species. Genetic diversity (including DNA sequence variation, copy number variants, deletions, and rearrangements) is the basis for adaptive evolution in all living organisms. Heritable differences among individuals influence how they interact with the physical environment and other species and how they function within ecosystems. Traits (e.g., height, fruit size, water needs, soil preference) influence survival and productivity of a plant, for example, via tolerance of temperature, pH extremes, drought, freezing, flooding, salinity, heavy metals, diseases, and a wide range of environmental conditions (Huenneke 1991, Hajjar and Hodgkin 2007). Many modern crops and some horticultural and forestry species have low genetic and trait variation due to various aspects of modern agriculture such as domestication bottlenecks, desired uniformity, high productivity, loss of traditional seed exchange networks, and consistent and known plant performance in high input agricultural systems. Genetically based variation in form, phenology, pathogen resistance, environmental tolerance, and other traits frequently exists in wild populations of cultivated species or in closely related species called wild relatives (Maxted et al. 2008, Hajjar and Hodgkin 2007). Incorporating genetic variation from wild populations or from closely related species into breeding programs and eventually into production could make cultivated plants more productive in highly variable or challenging environments (Threthowan and Mujeeb-Kazi 2008). Of course, natural populations rarely exhibit the final desirable whole plant phenotypes but can contribute valuable alleles and traits to breeding programs (Maxted et al. 2008; Khoury et al. 2010; McCouch et al. 2012). Currently minor and unimproved crops also have much potential for breeding and selection by utilizing genetic variation (Syfert et al. 2016). Collection, conservation, and characterization of genetic resources, including wild species, is increasingly recognized as essential for maintaining functioning forests and agriculture, providing useful genetic variation such as resistance to diseases and pests, and facilitating adaptation to environmental changes (Harlan 1975; Qualset and Shands 2005; Damania 2008).

Ex situ collections are repositories of seeds, pollen, or living plants designed to contain some proportion of the genetic variation of a species, usually a subset of what exists in natural populations (e.g., Volk et al. 2005, Cavender et al. 2015, Griffith et al. 2015) but sometimes including genetic variation no longer present in the wild (e.g., varieties no longer in use, geographic locations that have been extirpated). Landraces (domesticated, local ecotypes) sometimes contain more variation than what exists in the wild. Ex situ collections conserve and make available germplasm, providing an opportunity to study and incorporate the wide genetic variation of a species and its close relatives into agricultural systems (McCouch et al. 2012). Ex situ collections include botanic gardens, seed banks, seed orchards, breeding and provenance trials, and active restoration collections. The scope of collections varies immensely in number of samples and geographic coverage. For example, the International Rice Research Institute gene bank has more than 127,000 accessions (www.irri.org), while minor crops may have a dozen wild accessions. All collections face a common challenge – achieving the maximum useful genetic variation with the lowest number of accessions as a result of limited resources (space, time, money, personnel, etc.).

Box 8.1 Units of Genetic Variation that Can Be Conservation Targets
It is important to clarify units of genetic variation that are the potential conservation targets in ex situ collections (Fig. 8.1). Conservation targets for agricultural-based gene banks are usually at the sub-taxonomic level, for example, diversity is sought for a specific trait (e.g., aluminum tolerance, salt tolerance, disease resistance) or for broad population representation needed for contingencies in the future (providing option value). The most basal target unit is variants at a particular gene, called alleles – there may be from two to dozens of alleles for each gene. The next unit is the genotype, a unique combination of alleles across multiple genes (Fig 8.1a). A genotype can be assessed from a small number of genes up to the entire genome – in which case a genotype is the genetic constitution of an individual with respect to the alleles under observation. A genotype can also include a haplotype block, which is a continuous sequence of DNA, often inherited as a unit and not broken up by historical recombination, often long, and which may contain several or many genes. Alternatively the components of a genotype can be spread across a genome, called a coadapted gene complex. Note that a phenotypic trait may be based on a gene, a haplotype block, or a coadapted gene complex, combined with the influence of the environment. A maternal line is the next unit, composed of offspring from the same maternal plant (see Fig. 8.2). A population, the next unit (Fig 8.1b), is defined as a collection of genotypes, typically at a point in space and time. A population can thus also be defined by the frequencies of alleles or traits across all the interbreeding individuals in that location. The next unit is a lineage, which can include groups of populations or subspecies, species, genera, families, etc. This hierarchy of units of genetic variation for collectors to target reflects the natural ways that genetic variation is developed and maintained.

In this article we discuss the challenge of designing a sampling strategy that captures an appropriate amount of genetic and trait diversity into the ex situ collection. We will focus on designing seed sampling for a single species, recognizing that sampling across many species to ensure capturing phylogenetic diversity is another, related but different, problem (Griffiths et al. 2015). This chapter is organized in

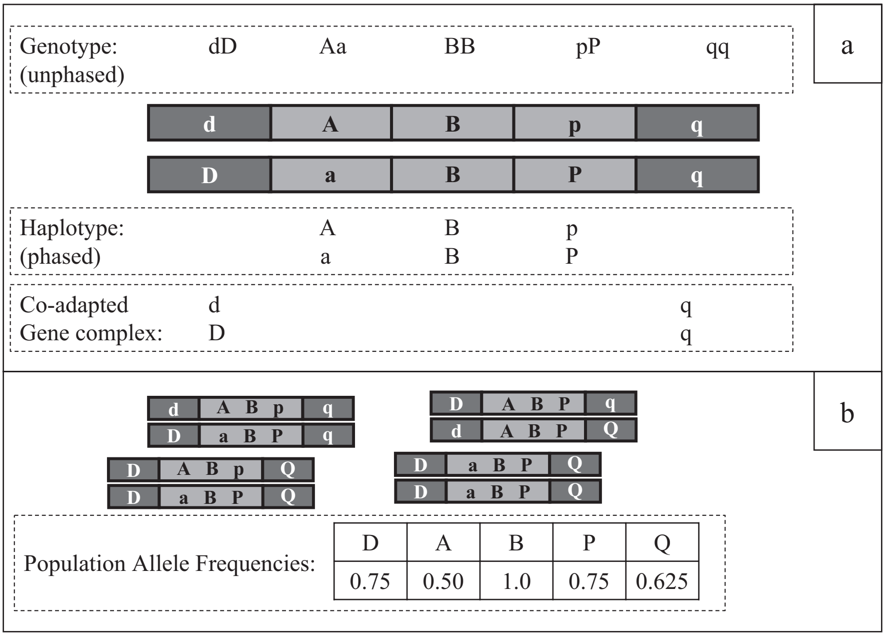

Fig. 8.1 Possible units of genetic variation that a collector can target. The linear shapes are chromosomes. (**a**) One individual is shown; (**b**) four individuals are shown. Each locus is represented by a block with a letter. Alternate alleles are capital and lowercase letters. A genotype is all the alleles in focus. Haplotypes are linear blocks in a chromosome, while gene complexes may be scattered. Population allele frequencies are calculated across several individuals

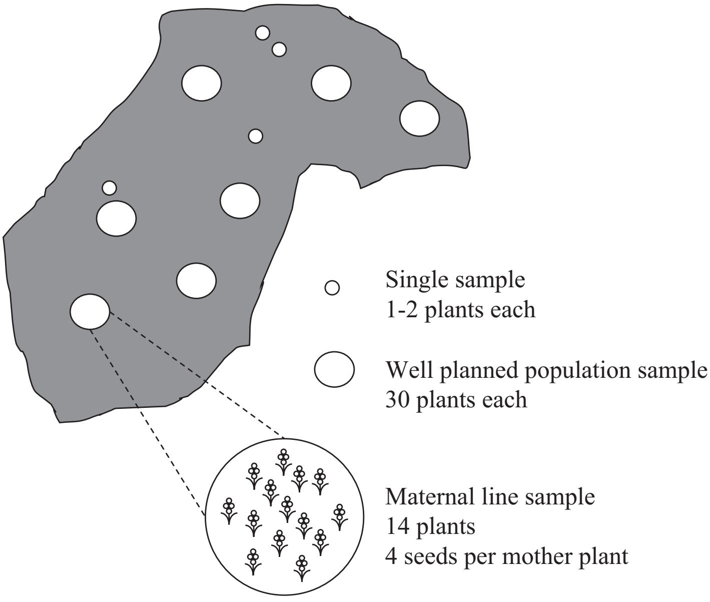

Fig. 8.2 Possible scope for sampling intensity and organization of samples across a species' range (gray shape). Single samples are often not systematic and have few individuals. Population samples are larger and more systematic. Maternal line collections are similar to population samples but with the added feature that each seed collected is traceable to a maternal plant (often to a GPS location)

sections regarding (1) intensity and organization of a collection, (2) minimum sample size rationale, (3) approaches to designing a collection, (4) practical considerations, and (5) future directions and needs.

8.2 Planning the Right Intensity and Organization of Sampling

Before designing a sampling strategy, a collector must determine the scope of their sampling and seed bank management effort. There are three general ways in which to collect samples, which differ in the amount of effort involved: (1) a single bulk sample for a species, often from a single population collection; (2) a multiple-population sample, which keeps and curates each population separately; and (3) a multiple-population sample, which further keeps and curates seed from each plant separately (i.e., separate by maternal lines). These three approaches correspond to the conservation targets explained in Box 8.1, the species or lineage, the population or locale, and the maternal plant or family, respectively. It is crucial to recognize that the methods by which seeds are collected will dictate the future research and breeding utility of ex situ collections, in perpetuity. For example, genomic, spatial, relatedness, and environmental data can be used by plant breeders to identify agronomically important variation (Neale and Kremer 2011). Briefly, maternal collections have the most potential utility for research and breeding but require the greatest effort in collecting and curating. We discuss here a framework to help collectors visualize trade-offs, analytical options, and limitations associated with their conservation focus and sampling techniques. We hope this will help collectors make informed decisions on selecting sampling strategies which balance logistical effort and resource allocation with potential collection utility.

Single-point collections of a species constitute the most basic level of collecting in terms of logistics, travel and site access; can be anywhere in a species' range; and sampling requirements are low -often one or a few samples, often at one site. For example, several CWR inventory studies have used a gap analysis methodology to analyze species overlap and diversity "hot spots" (Maxted et al. 2008, Ramierez-Villegas et al. 2010) which may provide logistical advantages of having multiple target species in a single location. However, sampling within hot spots may result in capturing just a fraction of species' diversity over its entire range and therefore not serve a conservation target below the taxonomic level. This sampling will also allow only few analytical options – mainly to identify the species and (combined with similar sampling from many other species) resolve taxonomic relationships (Hipp et al. 2014). With high enough numbers of sampling points, within-species genetic variation and genetic structure may be discernible, and in some cases spatial analyses of these collections can identify a species' environmental niche (see Sect. 8.3).

A collector may instead sample from a defined set of populations (i.e., groups of individuals that can mate and thus share a common gene pool or lineage), which are local sources of allelic variation. Sampling multiple populations is needed to capture the range of genetic variation within a species (Hoban and Schlarbaum 2014, Brown and Marshall 1995) and to enable many research analyses, and thus this is the level that many agronomic banks focus on. Some guidelines recommend at least 5 populations for rare species (Godefroid et al. 2011) or at least 50 populations for common species (Brown and Marshall 1995), while others focus on ecogeographic coverage where the emphasis is to cover the range of environmental conditions the species can persist in. Depending on sampling intensity, DNA analyses of these samples can reveal range-wide patterns in genetic variation (Hoban et al. 2010), recent demographic change (Excoffier et al. 2013), regional patterns of genetic differentiation (which may identify particular areas of interest for further collecting), and sometimes genetic differentiation by habitat which may be due to natural selection (Forester et al. 2016). Reintroductions using these collections can also reveal fitness of given source populations. Interpopulation sampling involves substantial pre-trip planning (choosing multiple well-spaced sites) and more complicated logistics (including timing) than single-point collections. In addition, progeny or other samples must be stored separately by site. At the location, sampling is usually limited to collecting sufficient numbers of individuals to capture the most common alleles that represent the taxon (Lawrence et al. 1995). Numbers of individuals per site and numbers of sites are determined in part by a species' taxonomy, distribution, breeding system, and genetic history, but many genetic analyses generally require 30 individuals per site (Selkoe and Toonen 2006, Hale et al. 2012). In contrast, niche models and genetic-environment associations benefit from many more sites but few individuals (<5) per site (de Mita et al. 2013). There are several existing publications to provide advice on designing the sampling strategy for genetic analyses which may accompany the use of the germplasm (Hoban et al. 2013, Landguth et al. 2012, Oyler-McCance et al. 2013, Lotterhos and Whitlock 2015, de Mita et al. 2013). A key point is that the appropriate sampling strategy for a genetic analysis may differ from the desirable seed sampling strategy which we focus on in this article.

The most intensive approach is to keep records of each individual seed lot. This sampling requires keeping maternal units separate and is often used by conservationists to conserve rare and endangered species or by researchers seeking to use the collection for scientific study (e.g., Project Baseline, http://www.baselineseedbank.org/). When these lines are planted out, each group of siblings will be known, which allows partitioning of trait variation due to genetics, and thus researchers can estimate broad-sense heritability for traits of interest which is useful for breeders (de Villemereuil et al. 2016). Researchers can also determine mating system such as rates of self-pollination by analyzing these seeds (Geng et al. 2008). A subset of maternal line collections are those where spatial location is recorded for each individual. This adds additional time and equipment needed in the field (a GPS unit) but can facilitate more analyses, such as fine-scale spatial genetic structure (Hoban et al. 2014, Vekemans and Hardy 2004), seed dispersal patterns (Hoban et al. 2012), local site history, and associations with local environmental variables (altitude, soil type, etc.). As an example, Arnaud et al. (2011) studied microspatial and temporal genetic structuring in weedy crop wild hybrid beets in northern France and identified a gravity-based seed dispersal mechanism and limited pollen flow among sites.

In summary, the intensity of sampling seed (including the collection scope, the amount of information collected, and the way of organizing the collection) involves trade-offs in time consumed in the field, time and space in collection management, and the usefulness of the collected samples. If resources are available, an intensive collection can reveal a wealth of information useful to managing wild populations and utilizing genetic resources.

8.3 *Minimum Sample Size*: Number of Populations and Number of Seeds

We now discuss the problem of how many populations to collect from. Because phenotypes of wild individuals do not predict precisely their usefulness in breeding programs and because future needs are not always known, exploring and collecting must be systematic in order to capture the widest range of genetic diversity, which may be captured via surrogates like environmental, geographical, or taxonomic diversity. Collections may especially benefit from genetically unique, highly diverse, or unexplored areas. Hybrid zones are another source of wide genetic variation. Early plant explorers recognized the value in diverse crop collections and traveled to remote or unusual environments to bring back plants and seed. For example, the organization International Plant Genetic Resources Institute (IPGRI), now called Bioversity International, undertook more than 1000 seed collecting trips in 136 countries from the 1970s to 1990s (Thormann et al. 2012). For rare species, conservationists recommend sampling at least 5 populations (CPC 1991), while thorough agronomic collections may seek to sample 50 or more populations (Brown and Marshall 1995). However, there are limits on the number of places that can be visited, and genetic models are being developed to help determine an appropriate minimum number of populations to sample from for a given species (Hoban and Way 2016, Hoban et al. 2018 see next section).

There are also limits on the number of seeds that can be kept. Since the 1970s, several authors have attempted to define a minimum sample size that should capture most of the relevant genetic variation. Using basic sampling theory, Brown and colleagues (Marshall and Brown 1975, Brown 1979, Brown and Briggs 1991, Brown and Marshall 1995) devised what is now one of the most common minimum sample sizes used by seed banks, the “50 sample” rule (reviewed by Lockwood et al. 2007a). In a randomly mating population, this sample size should capture all the alleles present in frequencies greater than 0.05. Of sampling guidelines for 20 major seed collecting organizations, the Brown and Marshall “50 sample” rule was cited or used in about two-thirds of them (Hoban and Strand 2015, Hoban unpublished data).

These simple guidelines for number of populations to visit and samples to take from a population are based on many assumptions that are not realistic for real plant populations (Lockwood et al. 2007b, Guerrant et al. 2014). Plants often exhibit complex population genetic structure determined by geography, population sizes, adaptation, hybridization, or recent demographic history. Sampling for conservation targets at the sub-taxonomic level must consider the distribution of genetic variation within and among populations (see next section). This variation is linked strongly to life-history traits, particularly dispersal and reproductive mode (Hamrick and Godt 1996). Genetic and trait variation patterns may also arise due to chance events during the colonization and extinction of local populations. The magnitude of genetic variation may vary across a species distribution, where both central and marginal populations vary in their connectivity and their habitat suitability (Hoban et al. 2018). Other things being equal, plants that disperse genes widely and frequently, through pollen and seeds, tend to be less differentiated (i.e., populations will be relatively similar). However, populations with high levels of gene flow can still exhibit substantial differences among contrasting environments or at different ends of their distributions (Ortego et al. 2012). In sum, some populations will contain more genetic variation than others, and some populations will contain unique genetic variation not found in other populations (Petit et al. 1998, Swatdipong et al. 2009).

In addition, the "sample" is often assumed to refer to a single plant, and previous guidelines have not provided advice on number of seeds per plant to collect nor how to sample optimally in space. A species' seed and pollen dispersal characteristics will influence how much seed to sample from each plant and the minimum distance between which plants can be sampled. Hoban and colleagues (Hoban and Schlarbaum 2014, Hoban and Strand 2015, Hoban et al. 2015, Hoban et al. 2018, Hoban and Way 2016) have recently demonstrated that sampling guidelines based on knowledge of species' traits and genetic structure can provide much more optimal capture of genetic variation than the simple Brown and Marshall guideline (see next section). However, robust guidelines for how to sample species with different biological traits are still in development (Griffith et al. 2015). Kashimshetty has used a similar simulation approach which extends these findings (Kashimshetty 2016).

In short, there is much research and rapid development on the question of where and how much to sample. In the next section, we explain methods that can help plan a collection, in order of the effort and knowledge needed. A clear trade-off will be apparent: sampling-design approaches that require the most knowledge will provide optimal genetic diversity coverage and the most benefit for using the collections. Although sampling from 50 plants remains a widely accepted, suitable starting point when the conservation target is a single population of an outcrossing species, more refined sampling guidelines can be developed for most species.

8.4 Approaches to Designing a Sampling Strategy

One approach to help determine where to sample is to use species' distribution modeling (SDM, also known as ecological niche modeling, ENM). A key challenge, and opportunity, in planning collections for maximum diversity is leveraging information on a species' current distribution and habitat suitability. Ecological niche models integrate climatic and landscape features which may be a suitable proxy for genetic diversity information when genetic data is lacking, as in the case of many CWRs. These models provide an estimated environmental "envelope" for where a species might be present. Operationally this is a reasonable approach that integrates geography, ecology, and climate, so a collector can estimate plant distributions and interconnectivity among sites and, hypothetically, can identify ecogeographic regions that contain unique ecotypes, traits, and presumably genetic variation (Segelbacher et al. 2010, Wang and Bradburd 2014). Locality information is usually recorded at herbaria adhering to Darwin Core standards (Wieczorek et al. 2012, Endresen and Knüpffer 2012) and accessible from the Global Biodiversity Information Facility (GBIF, www.gbif.org/) or the Integrated Digitized Biocollections (iDigBio, www.idigbio.org/). Species distribution models can also help predict where suitable habitat for species may exist in the future under a range of climate change scenarios (Keith et al. 2008).

Comparisons of the species potential range obtained with an SDM to samples that have actually been collected may reveal sampling gaps that should form the basis of future collection trips (e.g., Ramírez-Villegas et al. 2010; Khoury et al. 2015). This can be used to select sites from particular environmental conditions (Greene et al. 1999) or to evaluate how well an existing seed collection represents the species' actual distribution and thus where to sample next. This approach requires that a range map or many observations of a species' occurrence are available. It utilizes environmental data derived from georeferenced natural population localities which is used to build models of a species' ecological requirements and then to predict potential suitable habitat and geographic area (Kozak et al. 2008). For example, Castañeda-Álvarez et al. (2015) focused on wild relatives of sweet potato and calculated the proportion of their ranges as determined by SDM which are covered by 50 km diameter circles around known seed sample sites. There are numerous assumptions of this approach: that genetic variation is evenly distributed throughout the range, that 50 kilometers roughly defines populations or ecotypes, that the SDM is a good representation of a species range, and that climatic variables and geography are a major surrogate for genetic variation.

An alternative approach to determine how to sample is to first genetically survey the species. The collector will sample multiple populations at different scales (some nearby, some distant) and analyze neutral and/or adaptive genetic markers to obtain a dataset of estimated genetic variation that exists in the wild. The collector then uses a computational technique to subsample the genetic dataset repeatedly with different collection sizes until an optimal sample number is identified. Two examples are McGlaughlin et al. (2015) and Griffith et al. (2015). McGlaughlin sampled and genotyped multiple populations of the Santa Cruz rock cress, identified the most unique populations, and discovered by simulated subsampling that each island should be sampled in different fashion to best capture genetic variation (higher intensity sampling on one island than the other). Griffith et al. (2015) used genetic data to show that different sampling is needed for different populations and species (echoing conclusions of Hoban et al. and Kashimshetty et al. based on simulations) and also that collectors must visit over multiple years. The latter conclusion reflects the fact that different adults flower in different years and thus annual seed lots are genetically different. In sum, an initial genetic survey at low cost can be performed to understand distribution of alleles and genetic structure (in space and time), and thus inform the best seed sampling strategy. Such a study has an added benefit of providing knowledge on a species' distribution, phenological timing, and biology. Multiple collection trips over time enable comprehensive sampling, iteratively: initial collecting efforts provide information on the genetic structure of a taxon in the wild, and subsequent sampling can increase the completeness of an ex situ collection. Though performing an in depth genetic study is a good "gold standard" to determine existing genetic variation patterns and thus how to sample seed, historically it has been expensive and time-consuming. Approaches relying on geography and environmental variables (such as SDMs) have much lower cost.

Other examples of this approach for identifying unique populations or for tailoring sampling strategy include Caujapé-Castells and Pedrola-Monfort (2004), Gapare et al. (2008), Schoettle and Sniezko (2007), and Richards et al. (2007). This approach is similar to and thus shares methodology from the field of population prioritization (see Box 8.2) and minimum population size analysis, e.g., Kim et al. (2012).

The genetic diversity within a species is influenced by contemporary forces of the selective environment and historical features of past environments (Alvarado-Serrano and Knowles 2014), interacting with dispersal. These forces, combined with the influence of climate, environment, and biogeography, can have significant effects in structuring the variation that the collector is seeking. Discordance between niche models and population genetic structure is not uncommon and may reflect changes in land use and dispersal (Thormann et al. 2017). Accounting for these species-specific factors by including genetic data into distribution models may improve predictions about regions with potentially unique diversity and may help to forecast future vulnerability to climate change (Fordham et al. 2014), evaluate fitness and extinction risk in rapid climate change (Urban 2015; Anderson 2015), and better plan seed collections.

In the absence of a genetic survey (which will be often), an alternative to SDM is a simulation-based approach. In this approach, a species' traits are used to build a demographic-genetic model, which is then used to create pseudo-observed genetic datasets, representing genetic datasets we might expect to see in a species like our focal species. These genetic datasets are sampled in the same way that a real observed genetic dataset is subsampled, to test the effectiveness of different sampling strategies (Hoban and Schlarbaum 2014). This new approach is inspired by use of simulations to develop core collections as subsets of the total seed collection (Bataillon et al. 1996) and to manage regeneration of a collection (Richards et al. 2010). The advantages of this approach are the following: a genetic dataset is not needed, a large number of populations can be simulated (many more than could be sampled and genetically analyzed for some species), and the simulations can include rare alleles that wouldn't be captured in many realistic genetic surveys. The disadvantage is that a priori knowledge of a species' biology and distribution are needed and the approach is improved by more knowledge. Knowledge that can be incorporated into the simulations includes mating system, population sizes, annual or perennial habit, density, pollen and seed dispersal distances, age/stage distribution and transitions, reproductive success, and spatial distribution of populations (Hoban and Way 2016, Hoban et al. 2015, Hoban et al. 2018). Few species will have all of this information available, but if at least some information is available, a simple yet useful simulation model can be constructed.

The simulation approach can be used to design sampling strategies for broad classes of plants such as fragmented species' ranges (sample X seed for a species of a given class), to determine relative sampling sizes for classes such as self-pollinating vs. outcrossing (sample Y times more seed for a given class than another class), and to design specific strategies for species of high importance (sample Z seed for a specific species). For example, Hoban and Strand (2015) demonstrated that highly selfing, low-dispersal species may need total seed collections five times larger than outcrossing, high-dispersal species. Hoban and Schlarbaum (2014) demonstrated that when sampling many populations across a species' range (e.g., 16 populations), 25 samples per population will perform almost as well as 50 samples per population due to allele sharing among populations. Note that simulations can also optimize the eventual outplanting, organization, or other uses of a seed collection (Kashimshetty et al. 2012, Bataillon et al. 1996, Richards et al. 2010)

There is a major caveat to all of these approaches – they capture genetic variation in terms of number of alleles (genetic diversity in broad sense). They do not focus on alleles known to be under natural selection. Targeting alleles that are known to be of selective advantage is still a challenge. In spite of much work on understanding genes of traits and biochemical pathways (e.g., Scossa et al. 2016), in most cases we lack the knowledge of how most adaptive alleles are distributed among populations and whether survival and adaptation of individuals is due to single alleles, complex suites of alleles, or intricate gene by environment interactions. Sometimes alleles at single genes of importance are known to occur in clines (e.g., flowering time in aspen, Hall et al. 2007), and sometimes they occur in isolated pockets of populations. For example, Windham et al. (1998) found resistance to dogwood anthracnose in only one small population. Until we gain better genomic knowledge, we suggest that collectors focus on sampling a diversity of traits, geography, and environmental conditions, including isolated populations that have drifted or adapted in isolation. An issue is that there are currently no tools to help devise sampling strategies that incorporate these multiple factors simultaneously. However, collections focused on a particular dimension of diversity, such as resistance, can use knowledge about the biology of the trait (Khazaei et al. 2013) and suspected drivers of trait evolution. For example, pathogen resistance is likely to arise in areas where the pathogen load is high. Drought tolerance is likely where the abundance and timing of rain is limiting. In some instances the general application of gap analysis, with additional climatic, edaphic, and pathogen data, can be extended to identify ecogeographic regions within a species range that might have priority for collection to meet a targeted trait (Khoury et al. 2015).

Box 8.2 In Situ Genetic Preservation: Designing a Reserve

Effective conservation consists of both ex situ and in situ elements. In situ preservation has the advantage that the species can continue to adapt to environmental change via natural selection and can fill its role in an ecosystem, but the disadvantage that documented genetic material is not immediately or conveniently available for use (see Schoen and Brown 2001 for more discussion). The establishment of in situ *genetic reserves* (geographic locations which are chosen to optimally preserve the genetic variation of the species in its natural habitat) has similar goals and approaches as ex situ seed conservation. As with ex situ sampling, the choice of reserves may be made based on knowledge of genetic data or use of environmental variation and may include the most distinct, diverse, or isolated populations (Flower et al. 2018). There are additional considerations for in situ reserves, including political and social constraints such as current and future land ownership and use, possible threats to in situ populations, costs of management, and often multiple and sometimes conflicting goals. In situ reserves must also contain a certain minimum number of adult plants in order that the population will persist and evolve. The field of systematic conservation planning (SCP) has developed sophisticated methods that help to choose optimal reserve sites even with these many considerations (Margules and Pressey 2000). It is notable that these methods have been used for more than a decade for choosing sites that protect species diversity, but are only slowly being adapted for genetic conservation, even though the concepts are similar.

One example of in situ genetic reserve design is provided by *Dipteryx alata Vogel* (a common tree species native to the Brazilian Cerrado). Diniz-Filho et al. (2012) applied a SCP approach to choose the minimum number of populations needed to conserve all known alleles (using microsatellite markers), while also maximizing the amount of natural land cover preserved (a nongenetic constraint in the optimization problem) and considering genetic variation already preserved in a seed bank. Another example concerns the coordinated choice and monitoring of European forest genetic reserves of economically and ecologically important, as well as rare, species. The European Information System on Forest Genetic Resources (http://portal.eufgis.org/) has documented the location of in situ genetic reserves for 103 species in 35 countries, while the project LifeGenMon (http://www.lifegenmon.si) is developing protocols and means for monitoring these reserves. The multiple goals of these reserves include an even distribution across a species' range, coverage of known ecotypes and demarcated environmental zones, inclusion of Ice Age refugia and mixing zones, and consideration of known genetic variation (from molecular markers or provenance trials).

8.5 Practical Sampling Considerations

We discuss here other considerations for sampling: sustainable seed collection, accounting for seed storage and use, avoiding selective filters and bottlenecks as seed is handled, and sampling the wild population repeatedly over time.

It is important to preserve wild populations, and thus it is crucial to limit seed collection to ensure that the population will persist through natural regeneration. Menges et al. (2004) showed that safe levels of seed harvest can be determined via relatively simple population modeling. They showed that harvesting 10% of seed in 10% of years will rarely be detrimental, while harvesting 50% of seed in 50% of years is usually detrimental. They also showed it is better to spread out seed harvest over multiple years rather than intensively harvest most or all seed in one season. Meissen et al. (2017) investigated this same question using datasets on real populations that had previously been harvested over time. Similar to Menges' work and others (Turnbull et al. 2000), Meissen emphasizes that a species' traits are a large determinant of how much can be harvested and how often. Short-lived, non-clonal plants are especially sensitive to frequent harvest, while long-lived and clonal plants are more resilient. Some annual plants may be entirely reliant on each year's successful seed production. Way (2003) of the Royal Botanic Gardens, Kew, suggests a maximum of 20% of the available seed on the day of collecting, but "if any specific data is available on longevity, fecundity, masting, and establishment, this data should be analyzed to set more specific safe limits for collecting."

Once the initial minimum is devised, many factors that affect seed attrition should be considered. Way (2003) suggests that a minimum of 500 seeds (from a total of 50 different plants) is needed to prevent genetic drift. Additional seed is needed to allow for regeneration after long-term storage, as well as germination tests to monitor seed viability (e.g., 100 seeds to test every 10 years), distribution to users who request seed (e.g., 50 seeds every other year), and duplication in at least one other location in case of catastrophe. In some cases, collections must also be split into "active" (those available for request and use) and "reserve" (backup) collections, doubling again the minimum collection size. In total, Way suggests that 10,000 to 20,000 seed per population would make an ideal collection. Populations producing very few seed should be carefully assessed for their value, and the availability of more productive populations should be researched.

Basey et al. (2015) remind us that genetic variation is an important consideration throughout the seed collection, cleaning, regeneration, and production process, not just the sampling phase. They outlined ten important considerations to preserve genetic variation. These include taking care to not lose seed during cleaning or to size-select seed, using diverse germination conditions to ensure all seeds can germinate, replanting accessions whose attrition rates are high, and ensuring that subsequent seed harvests are performed throughout the growing season. The last recommendation is made because the timing of seed ripening is controlled to a large degree by genetics, and it is vital that a seed collection has a diversity of ripening times for future environmental conditions. To capture early-, mid-, and late-season genotypes will of course require multiple temporal collecting efforts in each spatial location.

Populations also change through time due to adaptation or to drift. Indeed, populations may respond rapidly to new pathogens or environmental change, which increases the frequency of some alleles while eliminating others, but this issue has not received much research from the standpoint of seed collections. After enough change has occurred, the original seed collection will no longer represent the current population genetic diversity, and thus a new collection should be made. Note that the original collection retains value, especially if it contains variants no longer existing in the wild, but the current population may have new variants of interest. Greene et al. (2014) examined two collections of a wild clover species made over time, in addition to a modern collection from the population. They found only small differences in the genetic makeup of the populations through time, using neutral markers, and concluded that collecting intervals greater than 15 years should be sufficient. This species has very large populations and is cross pollinated by insects, and thus genetic drift would be slow. On the other hand, Jensen et al. (2012) showed that it is important to monitor adaptive genetic variation over time to determine collection intervals, by identifying significantly different resistance to a fungal pathogen in barley collections over 15 years. Another example is evolution of wheat resistance to powdery mildew in France (Paillard et al. 2000). In general, response to selection (adaptation) can occur over relatively rapid timescales (Thrall et al. 2011, Hendry et al. 2011), though lags in adaptation are also common, with likely different consequences for annual and long-lived plants. No guidelines exist as yet, but sampling every 10 to 50 generations may help capture such genetic change.

Other authors have written about numerous other vital aspects of seed collection, such as monitoring seed viability over time (Walters et al. 1998, Schoen and Brown 2001, Richards et al. 2010). It is worth noting that approximately 10–20% of angiosperms have seeds classified as "recalcitrant" (Walters et al. 2013), meaning they cannot be stored by desiccation and cooling. Such seed collections will need to be kept as excised frozen embryos, living tissue cultures, or living plants in repositories like botanic gardens and arboreta. These methods are more intensive and expensive than traditional seed banking, and it is likely that fewer samples may be kept.

One of the most important but still unanswered questions for many seed collections is the degree to which collected seed should be used in the environment or location in which it was collected. Understanding local adaptation and its genetic basis continues to be a challenge for geneticists and restoration biologists (Hoban et al. 2016, Bucharova et al. 2018). A common assumption has been that a plant will be locally adapted and perform best in its source environment. While local adaptation (and the genes underlying it) continues to be documented, it has also been shown that many plants can perform better in environments other than their source. Indeed, much of production in agriculture and forestry relies on this fact. Local adaptation is most likely in populations of consistently large size, isolated from large amounts of gene flow, in novel or distinct environments for a long period of time. Small or recently established populations are less likely to be locally adapted, though some invasives do rapidly adapt. An increasing consensus suggests that plants can perform well at sites that are nonlocal and that mixing seed from different sources may give collections a better ability to persist in new or fluctuating environments (Havens et al. 2015, Vitt et al. 2010).

8.6 Recommendations and Future Work

We conclude with the following recommendations for planning wild seed collections with the aim of capturing high genetic variation of maximal utility for future use.

- Collectors should first determine the target genetic unit and the conservation focus of the collection, especially recognizing the benefit of multiple populations and (when possible) of collecting by maternal lines (each mother plant). Regardless of the approach, comprehensive spatial/environmental data needs to be recorded during collections to ensure that the seeds are usable to researchers and breeders.
- The minimum number of samples should be determined by a species' inherent traits and geographic distribution, which can be determined in advance with a relatively small desk study or modeling – an effort that will benefit in terms of an optimized collection that does not waste resources. The locations to sample can be determined in advance using niche modeling, a preliminary genetic study in which samples are collected and DNA is analyzed, or a simulation approach. These approaches are complementary in revealing unique or unexplored geography as well as unique genetics. We do emphasize that more knowledge is needed regarding how to sample species that differ in mating system, population sizes, density, pollen and seed dispersal distances, age distribution, reproductive success, spatial distribution of populations, and recent colonization history – This knowledge can be gained by simulation (Hoban and Strand 2015).
- Collectors should plan through all phases of sample storage and use in order to determine initial sample size, as per Way (2003), while also considering how much seed is too much, as per Meissen et al. (2017). Accounting for losses in storage and use can increase the suggested seed lot size by tenfold or more.
- Collectors must also return to populations periodically (perhaps every 5 to 50 generations) to capture new variation generated by drift and adaptation.
- Although genomic advances are revealing more genes under natural selection that may be useful for collectors to target in the very near future, currently geographic, taxonomic, environmental, and neutral genetic diversity are the best proxies for capturing genetic variation of broad utility for most species.

We hope these suggestions will make an ex situ collection (which may have a life span of hundreds of years) of wild species useful to researchers and breeders for near-term use and for many years to come, including for applications we cannot currently foresee.

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Chapter 9
Practicalities of Collecting Wild Plants in North America: Insights from the United States

Laura F. Marek

Abstract Successful collection of wild plant germplasm requires careful preparation. Based on the author's experiences in the United States, this chapter describes the process of preparing and executing explorations to collect wild plant germplasm in North America. An outline is provided, followed by more detailed descriptions of the steps of setting up an exploration framework, obtaining permits and permissions, and developing trip logistics. Establishing an exploration framework involves locating populations of the target species through compilation of herbarium voucher data and additional resources and through contacts with plant experts and other knowledgeable people. Most places in North America require permission or permits for any vegetation disturbance activities. Neither the United States nor Canada has an overarching national level permit; every landowner needs to be contacted. Mexico requires a national collection permit although the national permit does not grant access; therefore, every landowner needs to be contacted. The process of obtaining permits can take several months or longer. Travel logistics involve coordination and development of an efficient route based on population locations, the preparation of suitable maps, and the acquisition of appropriate devices and support equipment.

Keywords Exploration framework · Permit · Permission · Logistics · Herbarium voucher record

9.1 Introduction

As described in many of the chapters in this book, Canada, Mexico, and the United States are home to a wealth of plant species useful for development and improvement of a wide range of crops. Acquiring samples of this germplasm from the wild

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L. F. Marek (✉)
Department of Agronomy/North Central Regional Plant Introduction Station,
Iowa State University, Ames, IA, USA
e-mail: lmarek@iastate.edu

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is a process; this chapter provides an outline (see text Box 9.1) and discusses the steps involved, based in part on the experiences of the author, collaborators, and colleagues collecting seeds from wild populations in the United States for the US National Plant Germplasm System (NPGS) gene banks.

There are three components to any exploration to find and collect wild plant germplasm: the first involves setting up a framework of populations to sample, the second involves obtaining permits or permissions to access and make such collections, and the third encompasses travel and collecting logistics. Plant germplasm explorations are generally not random events; that is, it is usually not productive to simply drive or wander around hoping to locate populations of species of interest and randomly collect seeds or vegetative samples, in part due to permit/permission requirements.

Permitting can take a significant amount of time, from weeks to months or longer depending on the circumstances and agencies or organizations involved. The permitting/permission process should be underway as soon as population locations begin to be identified and will typically be ongoing with continued discovery of potential population locations. In most cases, it is not possible to collect germplasm anywhere in North America without a permit or some level of permission; it is also often not possible to obtain permits or permissions without specific information about population locations or areas to be explored. The type of permit (or whether sampling is allowed at all) can depend on the intended use of the collected material, including scientific research (the type of research may be a factor), accessioning in gene banks, documentations as herbarium voucher samples, immediate commercial application, or possible future commercial use. Permitting can also depend on the final destination of the samples relative to the sampling locations. International transport or exchange of collected germplasm is not addressed in this chapter; the topic is considered in the first chapter as well as in each of the three country chapters at the beginning of this book: Chapter 1, Wild Plant Genetic Resources in North America: An Overview; Chap. 2, Genetic Resources of Crop Wild Relatives: A Canadian Perspective; Chap. 3, Crop Wild Relatives in Mexico: An Overview of Richness, Importance, and Conservation Status; and Chap. 4, Conservation of Crop Wild Relatives in the United States.

Box 9.1 Planning Outline

1. Build a framework and identify extant populations
    (a) Herbarium voucher records, other public records, and mapping
    (b) Potential contacts
        (i) Herbaria and university departments and collectors of the herbarium vouchers
        (ii) Public land (federal, state, county, municipality) botanists and other specialists

(continued)

Box 9.1 (continued)

        (iii) Military lands
        (iv) Tribal nations
        (v) Nongovernmental organizations such as nature conservancies and natural history associations and amateur botany organizations

2. Identify landowners and obtain permits/permission
    (a) Permit contacts
        (i) Public land (federal, state, county, municipality) botanists and other specialists
        (ii) Military lands
        (iii) Nongovernmental organizations such as nature conservancies and natural history associations
        (iv) Tribal nations
        (v) Private lands
    (b) Maps, county assessor records, and online portals
    (c) Permits/permissions: access, sampling, and use
    (d) Highway right-of-way access
3. Logistics
    (a) When: is this really the year to collect
        (i) Time of year: appropriate vegetative sampling or mature seeds
        (ii) Current year weather conditions
        (iii) Other environmental considerations
    (b) Where: potential routes
        (i) Escort may be required; routes, locations, and timing predetermined in a permit
        (ii) Multiple maps are recommended: hard copy and digital
        (iii) Be prepared; cell phone service and satellite contact may not always be available
        (iv) Confirm open roads, lodging, and fueling options
    (c) Equipment
    (d) Sampling
        (i) How to sample
        (ii) Sample viability
        (iii) Minimum population size and maximum sampling amount may be determined in permit(s)

(continued)

Box 9.1 (continued)

- (e) Recording “passport data” (population location and associated data)
    - (i) Collection/sampling date and collectors
    - (ii) Exact location: latitude and longitude coordinates and elevation from GPS device
    - (iii) Describe how to get to sampled location: trails, roads, highways, and nearest town
    - (iv) Describe the location and associated vegetation
    - (v) Image the location and population and species details if allowed

4. Flexibility: be prepared

9.2 Building a Framework

9.2.1 *Herbaria and Public Records*

A preliminary infrastructure of potential sampling locations can be based on information obtained from herbarium voucher records and similar public sources, such as published research about the species of interest and natural area floral surveys and floras. Many herbarium voucher records are available from online databases managed at the herbaria or in regional cooperative ventures such as the SEINet (Southwestern Environmental Information Network, managed at Arizona State University), whose records include six Mexican herbaria, and the CPNWH (Consortium of Pacific Northwest Herbaria, managed by the University of Washington Herbarium), whose records include five Canadian herbaria. Many online herbarium searches offer sophisticated mapping functions which display selected records on a map. Some herbaria have no records online, and others have only a portion databased. Contacting the herbarium directors can help determine if a personal visit would provide significant additional location information. The New York Botanical Garden maintains a database of the world’s herbaria, Index Herbariorum, which can be easily searched online (http://sweetgum.nybg.org/science/ih/) to locate region-specific herbaria, although the explorer should expect that most herbaria will have vouchers for populations not in their immediate geographic area.

Many voucher records are decades old and are often only useful as evidence that a species could be expected in a geographic area. However, the author has found populations at locations described in voucher records 70–80 years old; success depends on land use history and whether the species is annual or perennial. Many voucher records predate handheld Global Positioning System (GPS) devices, and associated latitude and longitude values were likely determined from a map or, in the United States, as a conversion from recorded Public Land Survey

System data (section, township, range). Therefore, these coordinate values rarely indicate exact sampled population locations, and their usefulness should be assessed by comparison with verbatim voucher location descriptions. Many collections were made along roads and highways, but in the intervening years, roads may have been rerouted, renumbered, reconstructed, become impassable, and/or been eliminated. Consulting older and multiple maps in combination with online searches of highway numbers/names can be useful in sorting this out. Based on the author's experience, less than half of the populations described on voucher records are likely to be located, significantly fewer if relying solely on roadside vouchers; however, depending on the species, an explorer can expect to discover at least a few populations in appropriate habitat areas that were not previously vouchered.

A continuing trend at herbaria is to attach images of the vouchers to the database records. Looking at the vouchers themselves can be useful because sometimes information is present that has not (yet) been entered into a database, including collection date and plant phenology, population size, whether or not the species identity has been confirmed by an expert, and location description. On modern records, the collector's name can be useful in providing a potential contact about the species of interest.

In some cases, identifying relevant, unrepresented areas to explore is a goal. Geo-climatic data layers can be overlaid onto location maps developed from voucher specimen data and other determined population locations. The geo-climatic characteristics can indicate other geographic regions within which to target a scouting trip and/or herbaria and natural areas to contact for additional information. Precipitation in combination with soil data are useful indicators, as demonstrated in Kantar et al. (2015). A scouting trip can confirm locations on which to focus efforts to obtain access and use permits, although in the case of herbaceous plants in active use areas such as roadsides, there is no guarantee that a population will be intact and not mowed or otherwise modified upon returning later in the season. For roadsides, there is the additional consideration that established vegetation could be the result of restoration/revegetation efforts after roadwork and, therefore, of unknown origin. The states' departments of transportation or the equivalent can sometimes provide information about seed mixes used for roadside restorations.

9.2.2 Local Contacts

Preliminary maps prepared before consulting with local experts provide a basis for discussion about whether extant populations can be expected in specific areas during the sampling year. The herbaria are often good places to start to identify local contacts. Staff at these institutions are experts in a number of genera and/or often will be able to recommend other contacts, people, and natural areas.

In addition, scientists knowledgeable about species of interest can sometimes be discovered by searching university botany/biology department directories in geographic regions of interest and reading about the scientists' specialties. Botanists and other specialists are associated with the National Forests, the Bureau of Land Management (BLM), and the National Wildlife Refuges, as well as state, county, and municipality forests, parks, and other natural areas, such as Natural Heritage properties. The water management districts in Florida manage several million acres of public lands with associated natural resource specialists. Military installations in the United States cover more than 10 million acres, and many have environmental and natural resource divisions in their Directorates of Public Works, with biologists/botanists on staff. In the author's experience, visits to military lands require an escort and most require a safety training session or briefing before any field work is undertaken. Even if the biologists associated with public lands are not knowledgeable about a species of interest, they represent a first contact with whom to start a permitting process. Many tribal nations have an environmental resource department or its equivalent with associated botanists/biologists; again, these individuals can be the first contacts in the permitting process, whether or not they know about species of interest. Some tribal nations are willing to allow access to their natural resources depending on proposed uses. Various nongovernmental organizations (NGOs), such as The Nature Conservancy (TNC), are focused on natural resource conservation, and their protected lands can provide a great resource in specific areas. Generally TNC has associated botanists/biologists who may be knowledgeable about species of interest or, again, who can be a first contact in the permitting process. In many areas of the United States, serious amateur botanists who can be reached through state native plant organizations and similar groups are often willing to share information about populations and current year conditions.

Taking the time to talk directly with people is often necessary. The author has found that a combination of telephone contact and electronic access is the only way to make efficient progress. There are still valuable contacts and organizations that do not rely on email for communication. In addition to population information, local contacts may provide year-to-date weather conditions and their effect on wild plant populations, as well as information about access issues.

9.3 Identify Landowners and Obtain Permits/Permissions

9.3.1 Permit Contacts

As mentioned in Sect. 9.2.2, local contacts will often be a first contact in a permitting process. The permitting process has changed within numerous agencies over the years, and, anticipating future changes, specific details and agency contacts are not provided here. Googling the agency, region, district, state, county, and department and searching for permitting often provide the needed information. Sometimes the most efficient way to locate a permitting process is to make phone calls, although the actual process may end up being more or less online; typically at least, the initial permit is electronically available. It is not uncommon, however, to be required to check in with a local office in person when on an exploration, and some agencies require permits to be signed and picked up in person.

9.3.2 Landownership

The first step in permitting or obtaining permissions is the identification of landowners. In some cases, it is very obvious that a record refers to a roadside, a national forest, or other properties that have formal permitting processes in place. However, there are pockets of private land within many of the larger US federal properties, and access is not covered by the federal permit. Generally, detailed BLM and National Forest maps are available to help assess if a private property owner should be identified. Also, herbarium vouchers do not necessarily indicate landownership, and all of the roads in gazetteers and on Google Maps and other online map programs may not be public, with the result that a roadside location may in fact be on someone's ranch or be behind a locked gate on a hunting preserve or a forest plantation. Natural Heritage Areas are usually state organized, frequently under state-managed natural resource departments or similar agencies, but they often encompass private property, and access can involve special permissions/escort.

When a location clearly maps to private land in the United States, landowners can often be identified using the Beacon and qPublic.net interactive public access portals, which allow users to view county and city information, public records, and Geographical Information Systems (GIS) online (22 states participating as of October 2017). County assessor records are also generally available online. Sometimes it is possible to obtain a phone number or other contact information from these resources or by Googling the owner's name; sometimes it will be necessary to write letters to the property owners. Landowners must be informed of the purpose of the plant sampling/collecting and how samples would/could be used when requesting permission.

9.3.3 Permits/Permissions

Permitting in the US National Forests (USFS, Department of Agriculture) is sometimes region wide (9 regions, USFS) or forest wide (159 forests, grasslands, and national recreation areas) but commonly is handled by individual districts within forests (most forests have at least 2 districts; several have as many as 9). The BLM (12 regions, Department of the Interior) usually issues statewide permits with a requirement to check in with local offices when in those areas. National Monuments may be under the jurisdiction of one of the several federal agencies (BLM, USFS, or the National Park Service (NPS)), are sometimes co-managed, and may require a National Monument permit that is issued separately from the primary agency permit. The NPS has an online permitting system organized by park through the National Park Service Research Permit and Reporting System (RPRS). A research proposal or study plan is required to begin the process. Based on NPS permit conditions (March 2017), it is not possible to include germplasm collected in the National Parks in gene banks that distribute without restrictions, but other research uses are possible. Ample time is needed for permitting; the process can take 3 months or longer.

Permits/permissions to sample populations of common species are generally straightforward. Species on the US Fish and Wildlife Service's (USFWS, Department of the Interior) Threatened and Endangered Species list require special permitting through the appropriate USFWS region (eight regions, USFWS). Public lands on which listed species are found will not grant access permits for those species until there is a permit from the USFWS approving the research plan. In the author's experience, NGOs and tribal nations also require the USFWS permit to be in hand before considering access to any threatened and endangered species populations on their lands. The permitting process for threatened and endangered species sampling often takes up to a year, and permission may be refused or the request modified. It is common for a land manager escort to be required when collecting from threatened or endangered species populations. States have separate rare species lists and may restrict access and/or use. It is the explorer's responsibility to know which, if any, species of interest are threatened or endangered (federal) or rare (state); often state and federal agencies will include lists at the time of permit initiation.

Restricted areas within public lands, such as the National Monuments within the BLM and/or National Forests and wilderness areas within any public land, require separate permitting. In the case of wilderness areas, motorized access is almost always prohibited, and sampling may not be allowed. If a research plan includes soil sampling or other land disturbances, a separate permit can be required, especially if the activity is taking place in areas where the proposed activity could affect archeological sites or other artifact sources. Some agencies require liability waivers as part of their permitting processes (e.g., TNC and Water Management Districts in Florida), and some require certificates of insurance (e.g., Florida Water Management Districts and some states for highway right-of-way access). Most have a reporting requirement after the completion of research/sampling.

9.3.4 Permission to Collect

During the permitting process, it is necessary to be clear about the purpose of the sampling, what is being sampled/collected (e.g., seeds, tissue for DNA, whole plant sample for voucher record and/or other purposes, soil for analysis), and indicate any known potential future uses of the germplasm. If samples are intended for accessioning into a genebank and/or if location-related information will be posted to a public database, the landowner must understand and approve (true for all categories of landowners).

9.3.5 Highway Right-of-Ways

Because of ease of access, many herbarium voucher/collection records have been made along roads and highways. In the United States, each state controls state and federal highway right-of-way access within that state through its Department of Transportation (DOT or its equivalent). Most states require a permit/permission to access to highway right-of-ways under their jurisdictions, often termed an encroachment permit, although the specific office involved varies by state. Generally, regulations concern safety for both explorers/collectors and passing motorists, not the specific plant germplasm. Permits are usually obtained from the individual state transportation district/regions that cover the geographic areas from which access would be required. The number of state districts/regions varies from one each in Delaware and Rhode Island to 25 in Texas. Most states require some level of personal protective equipment (PPE) be worn when working in right-of-ways, most commonly ANSI (American National Standards Institute) type II vests. In addition, many states require right-of-way users to post a certificate of insurance. The DOT personnel, contacted during the time period this chapter was being prepared (September 2016–September 2017), indicated that Louisiana, Oregon, and South Carolina did not allow state and federal highway right-of-way access for vegetation disturbance, although an exception for state/federal agencies could be possible in Oregon. The DOT personnel in seven states (Maine, Mississippi, New Hampshire, Oklahoma, Rhode Island, Vermont, Wisconsin, and West Virginia) indicated that a formal permit or permission was not required or a formal process was not in place for permitting for vegetation disturbance in highway right-of-ways; nevertheless, most required district/region notification of any activity, as well as use of PPE. The DOT personnel in the remaining 39 states indicated that a permit or permission to access state and federal highway right-of-ways was required. Some states have a fee structure for their permitting process, and generally DOTs are interested in specific locations and dates. Several months may be needed to acquire any required roadside permits/permissions. County and municipality roads are not under state jurisdiction and each county and city or town needs to be contacted individually regarding roadside access. Commonly, property ownership of lands adjacent to county roads extends to the middle of the road; there may not be a public right-of-way per se. Usually counties do not have formal permitting systems; the road commissioner or equivalent handles permissions. As mentioned in Sect. 9.2.2 above, established roadside vegetation could be the result of restoration/revegetation efforts after previous roadwork. The DOTs or their equivalent can sometimes provide information about seed mixes used for roadside restorations.

9.4 Logistics

9.4.1 When (Is This Really the Year to Collect?)

The best time of year for sampling depends on the basic biology of the taxa/genera of interest and the type of material being sampled (i.e., mature seeds or vegetative samples). Determining when a species will flower in its native habitat and when mature seeds will be developed is part of preliminary planning efforts by the explorer. If populations need to be sampled in the fall, hunting season restrictions can affect PPE requirements and access (sometimes not allowed). Searching for plant populations while listening to gunshots near and far during the hunting season can be unnerving. Permitting contacts can provide information about potential biotic and abiotic hazards if specific information is not on websites or provided with the permit.

Current year weather conditions in the area of interest affect the feasibility of a successful exploration. The US National Weather Service provides useful online tools such as the Advanced Hydrologic Prediction Service, which can visualize year-to-date precipitation data. If a severe drought is affecting population survival, land managers and other experts may suggest postponing an exploration. Late (or early) freezes can affect whether or not living germplasm or mature seed can be harvested during planned travel times, as can floods and hurricanes. Weather can also affect route planning, as discussed in Sect. 9.4.2 below. Other environmental considerations include forest fires, especially in the western United States/Canada, which can restrict access and/or close areas and roads.

9.4.2 Where (Potential Routes)

Sometimes an agency/organization escort will be required especially if a federally listed species is being sampled; therefore, schedules will need to be coordinated, and routes and locations can be predetermined. An explorer should be aware that it is still possible to be in areas with little or no cell phone service due to isolation and/or to limitations due to service provider coverage. Weather and terrain can affect satellite coverage and GPS accuracy. GPS units can track routes and indicate coordinates, so the explorers can know when a certain latitude and longitude has been reached, but cannot necessarily effectively guide them to isolated rural locations. In addition, sometimes navigation results are simply wrong. It is prudent to have actual paper copies of area gazetteers and atlases and to not depend solely on electronic devices. Multiple maps are also useful because old voucher records often reference landmarks (churches, cemeteries and the like) on topographical maps which are generally not indicated on online maps nor in the gazetteers. Many maps can be downloaded onto digital devices such as GPS units, cell phones, and/

or tablets prior to travel, and Google Earth can cache images so that an Internet connection is not necessary for field viewing of your maps. In addition, there are mapping apps, such as Collector, Mappt, and others, which can function with satellite connectivity in the field and provide navigation as accurate as the maps downloaded to a device and/or voucher and other sourced latitude and longitude data. The technology has changed rapidly in the past few years and continues to evolve; therefore, specific digital device and software recommendations are not made in this chapter.

The collection route depends on where there are open roads, lodging, and fueling options. A broad range of factors will affect road status, including construction and detours (states have generally current websites detailing construction areas; it is worthwhile to check roads in areas of interest), weather (precipitation can make roads temporarily impassable or wash them out), and forest fires. Heavy rain from thunderstorms routinely makes unpaved roads in the Southwest impassable, sometimes for the rest of the season, and storm surges from hurricanes can flood roads and submerge coastal and near coastal plant populations. It is reasonable to spend time checking into this kind of information when preparing for exploration travel. There are various state and federal websites with information about active forest fires, which are a frequent concern in the West and Southwest during late summer and fall. For example, the California Department of Forestry and Fire Protection website, CAL FIRE, maintains a map of active fire locations in California that includes fires on federal forest lands.

The time of day when passing by/through urban areas should be considered to avoid times of heavy local work traffic. In some areas of the western United States, county roads may have higher speed limits than the main highways, although typically county roads have much lower speed limits (45 mph is common in the Southeast); speeds slow enough to allow observation of roadside vegetation are even lower. It is sometimes difficult to determine if a road is paved, and roads can be slow due to topography regardless of a posted speed limit. A high-resolution look at a route on Google Maps or similar online mapping service prior to travel will allow for much better time/distance estimates than an atlas or gazetteer. Some highway water crossings involve ferries, not bridges, and are indicated on maps by either a thinner, possibly different colored, line or no connecting line across the body of water. It is important to determine if and when a ferry is operational and to have an alternate route planned to accommodate ferry breakdowns (especially, the Utah State Highway 276 ferry crossing of Lake Powell).

It is worthwhile to spend a little time researching lodging options in potential end of day areas. During the fire season in California and other western states and in Canada, hotel rooms may be very hard to come by if there is an active fire anywhere in the vicinity; they will be occupied by fire fighters. Hotel rooms are also scarce in college towns during home football weekends or graduations and in locations during music and other festivals and special events. Railroad, highway construction, or utility work and seasonal tourist travel can tie up the rare hotel in a rural area of choice.

Fig. 9.1 Collecting wild sunflowers (*Helianthus* spp.). (**a**) BLM permitted collection location, collecting seed heads from a *Helianthus anomalus* population along the Hole in the Rock Rd, Grand Staircase Escalante National Monument, southeast of Escalante, UT. Photo: N. Harvey. (**b**) US National Fish and Wildlife Refuge permitted location, labeling sample bags of *Helianthus petiolaris* subsp. *canescens* along the El Camino del Diablo, Cabeza Prieta National Wildlife Refuge, southwest of Ajo, AZ. Plant press with herbarium voucher specimens adjacent to sample bags. Photo: L. Marek

9.4.3 Equipment

Depending on potential route(s), a four-wheel drive vehicle may be required; if so, be sure to reserve ahead if renting a vehicle.

Required equipment will vary depending on purpose, genera/taxa, and permit conditions, and there is no attempt to cover all possibilities here. Figure 9.1 illustrates some of the standard equipment in use on fine weather days during explorations for wild sunflower seeds. Careful thought should be given to what the exploration involves and plans made accordingly. Basic supplies can include bags for samples (paper is usually better than plastic for seeds), marking pens, clippers, gloves, equipment to ensure sample survival if necessary, and a plant press and supplies for vouchering. Weather-related needs include carrying a waterproof jacket or poncho, knee-high boots if bog or stream encounters are possible, and rain pants. The boots can also be protective in areas where venomous snakes might be encountered, and both boots and rain pants are invaluable early in the day in regions where humidity is high and morning dew is heavy. Wet samples are possible and require the availability of extra bags and newspapers upon which to spread samples for drying. Standard safety supplies, especially if traveling in remote areas, include extra water and snacks, a basic first aid kit, insect repellant, sunscreen, and hats. Disease-carrying mosquitos and ticks are a concern in many areas; include protective measures, both repellant and appropriate clothing. A GPS unit or a smartphone with a GPS chip is necessary to indicate exact location data and is useful for navigation. Whether using a traditional camera or a cell phone, a camera for imaging plant details, populations, and surrounding habitat is an important equipment consideration. The equipment of choice for recording passport data is briefly discussed in Sect. 9.4.5 and may be paper and pencil, electronic tablet, field hardy computer, or other digital device.

9.4.4 Sampling

It is incumbent on the explorer/collector to proceed responsibly so that the long-term survival of a population is not affected by seed or vegetation removal. This is a priority consideration for all wild plant sampling and research explorations.

Sampling strategy varies by plant type, species, population size, and research purpose. Based on the research purpose, the collector decides whether to bulk samples from individuals within a population or to keep samples separate. A primary consideration should be appropriate short-term storage of the collected germplasm so that viability is maintained for the duration of the exploration. Random/irregular sampling across a population is often appropriate when sampling for a genebank, and whenever diversity is high between individuals in a population, more individuals should be sampled. However, if high within-population diversity is observed, one should consider whether the population represents a hybrid swarm or zone, the probability of which varies considerably by genus. If more than one closely related taxon from the same genus is in close proximity, at the very least, this information should be recorded in the passport data. Chapter 8 in this book discusses sampling strategies and theory; regardless of theory, usually a minimum population size is established during the permitting process (small populations usually cannot be sampled), as well as a maximum sampling amount.

9.4.5 Recording Passport Data

During any sampling activity in the wild, the explorer/collector should record as much information as possible about a sampled population/location in addition to making a voucher record, without affecting the efficiency of the overall journey. Regardless of research purpose, the collection date, the participants, and an exact location and elevation should always be recorded. A good quality GPS device is the most accurate means of obtaining latitude and longitude data; an appropriate datum must be selected and recorded along with the device model and manufacturer. Cell phones with GPS capability can also provide latitude and longitude; phone model and app used must be recorded. Comparing phone results with a good quality GPS can be informative before deciding which unit to routinely employ. A detailed description of how the collection location was accessed, trails, roads, highways, and nearest town, provides geographic context and provides a comparison for error checking of mapped GPS coordinates. Future use of collected material and/or its related data will be more useful if additional information is recorded. Basic passport information includes a habitat description (e.g., roadside, forest, open forest, meadow, etc.); the slope and aspect of the site; information about the soil type, as well as associated vegetation; phenology of the sampled populations; population size; and the (approximate) number of plants. The Food and Agriculture Organization of the United Nations (FAO)/Bioversity has developed a listing of multi-crop passport data descriptors (Alercia et al. 2015), which may be relevant for specific collection applications. Some phone apps will indicate aspect and slope of locations in addition to GPS coordinates and elevation. Images of the location and the population are very useful because, in fact, a picture is worth a thousand words. However, in some locations, imaging may not be allowed or may be partially controlled (e.g., Wyoming roadsides, some tribal nations, some military lands, some private lands).

9.5 Flexibility

Despite all of the structure suggested by this outline, explorations always require a certain amount of flexibility.

Current year population information may be received well after a permit application is submitted or even after the permit is received, as the process of contacting local experts is ongoing. In addition, once in the field, a fabulous population or example of a species of interest may be observed in an unexpected location; whether it is possible to collect from it depends on the permit. These kinds of possible situations should be discussed when applying for permits/permission. Ideally, permits can be set up to have some flexibility for both location and genus/taxa. One possibility is to establish the permit using generic names and not indicate species or to include all potential species for a geographic area based on known ranges.

As mentioned in earlier sections (Sects. 9.4.1 and 9.4.2), weather and other environmental factors such as forest fires may be a significant factor in the success of an exploration and may result in the need for a last-minute reorganization or rerouting. Land managers may suggest a postponement due to poor population or land/road conditions. A change in activities at a military site may make an area not accessible the day of planned access.

During more than a dozen explorations, the author has found personnel in the United States at all agencies and organizations, and for the most part, local people encountered at sampling stops, to be helpful and accommodating. In only one case did a veiled threat make it seem prudent not to pursue an off-road public lands collection location. Occasionally dogs have discouraged random searching, and during one exploration, feral hogs in South Florida encouraged the author and co-collector to call it a day, rather than explore a beach area in rapidly falling darkness. Other types of animal hazards can exist; it is prudent to ask about possible issues when applying for permits.

9.6 Collecting Wild Plants in Canada and Mexico: Some Generalities

9.6.1 Canada

The processes described for collecting germplasm in the United States are generally applicable in Canada. Similar to the United States, Canada does not yet have federal laws or regulations specifically concerning access and benefit sharing; therefore, overarching national permission to collect is not required and only landowner permission is necessary. Also, as in the United States, there are multiple land agencies to contact. Canada has a national park system with jurisdiction over parks, national marine conservation areas and a national landmark, as well as a National Wildlife Service with jurisdiction over National Wildlife Areas and Migratory Bird Sanctuaries. Provinces in Canada have autonomy and more control than states in the United States. Most forests in Canada (90%) are under provincial/territory jurisdiction; there is no national forest system per se, but forests on national lands, such as parks and military installations, are under federal control. Each province and territory also manages and maintains protected lands as parks, ecological reserves, and heritage parks. Permits/permissions are obtained from landowners. Scientific research, including sample collection, in the territories (Northwest Territories, Nunavut and Yukon Territory) is governed by specific legislation and requires a license. Indigenous communities (First Nations) have jurisdiction over their lands and must be individually contacted. Indigenous and Northern Affairs Canada, a federal department, can provide initial guidance about how to proceed. As in the United States, some First Nations have developed protocols related to the genetic resources on lands they hold. There are also federal military lands and NGOs in Canada, such as The Nature Conservancy, which all require separate permitting. Similar to the situation in the United States, permits for collecting in highway right-of-ways are handled by the provinces or territories.

9.6.2 Mexico

In general, the processes described for the United States are also applicable in Mexico. One major difference from the United States and Canada is the requirement in Mexico for an overarching national permit which gives permission to collect. In addition to the national permit, an explorer must have permits/permission from individual landowners such as the national parks and biosphere reserves; from other national conservation areas including nature sanctuaries, protected areas, natural resource areas, and nature sanctuaries; and from land that is part of an ejido (village based collectively owned and managed lands which encompass about 50% of the land in Mexico) or privately owned property. As is true for some categories of permits in the United States and Canada, obtaining necessary permits can take much more time than the actual exploration. At the present time, the national permitting process to collect germplasm in Mexico for all use categories is under revision, and there is not an established legal framework for the exchange of ex situ germplasm (Chap. 3). The National Genetics Resources Center (Centro Nacional de Recursos Geneticos within the Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias) is a resource for up-to-date information as a process is being established. Travel in Mexico can be difficult, and it is advisable that a collector has good command of the Spanish language or travels with a collaborator who speaks Spanish well.

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Chapter 10
Genebank Conservation of Germplasm Collected from Wild Species

Christina Walters, Christopher M. Richards, and Gayle M. Volk

Abstract Crop genebanks are tasked with maintaining genetic resources that support agriculture. They must keep a diverse array of samples alive for decades to centuries. Controlled conditions within the genebank are necessary to maintain quality and ensure consistency of the sample through time. Challenges for providing quality and consistency increase with samples that are mostly unstudied and highly heterogeneous and respond in unpredicted ways, as is the case for samples collected from the wild. The task of genebanking will be facilitated by better definitions of the "conservation target," meaning the level of diversity that the sample is intended to represent. With that definition, collectors will have better knowledge of what and where to collect – and when to stop – and "fit-for-purpose" samples will be preserved. Major uncertainties persist about the life expectancy of the sample and whether genebanking imposes genetic shifts. Standards have been recommended by the international community to ensure lasting quality of samples despite a large number of unknowns. We believe some of these standards will be counter-productive or unobtainable for wild-collected samples, and we have offered alternatives that stress documentation so future genebank users can predict whether a sample will suit their needs.

Keywords Conservation target · Cryopreservation · Germplasm · Longevity · Preservation · Propagule · Sampling · Storage · Ex situ conservation · Genebank

10.1 The Challenge of Ex Situ Collections: Maintaining Wildness in Captivity

North America's rich flora has the potential to contribute genes to make our crops more resilient to disease, pests, and weather extremes. The unique features of many North American taxa also offer the opportunity to find sources for new products and

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C. Walters (✉) · C. M. Richards · G. M. Volk
USDA, Agricultural Research Service, Center for Agricultural Resources Research, National Laboratory for Genetic Resource Preservation, Fort Collins, CO, USA
e-mail: christina.walters@ars.usda.gov

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better nutrition or healthier, more sustainable landscapes. Many of the plants described in this book are congeneric with plants commonly used in agriculture. For these, breeders will select the genes of interest from the wild relative and assimilate them into modern cultivars, leaving behind undesirable genes that contribute to a weedy phenotype. The breeder's job is facilitated by low barriers to interspecific hybridization incumbent with wild species that are closely related to domesticated species (i.e., crop wild relatives, CWR). Similar concepts apply to other wild species that may offer new products or have greater resilience in a changing landscape (Urban, 2015). In these cases, modern-day domestication efforts may be invoked and provide an important reminder of the reservoir of services that wild plants provide, bringing new opportunities for economic growth, ecological sustainability, or aesthetic sensibility.

It makes sense to collect North American genetic resources in genebanks and make them available to agriculture, conservation, engineering, and scientific disciplines that explore biological diversity and the environment. We envision collections of genetic resources as an inventory of "nature's solutions" to tough environmental problems. Genebank users will sift through these collections for patterns of diversity or traits of interest. Therefore, genebanks must be careful not to "edit" nature's work in case it masks or removes the very trait that was sought. This creates a real challenge because the highly controlled conditions that genebanks must use can create strong pressure for plastic living systems to adapt and become domesticated. The signature of domestication is particularly recognizable in seed traits (Fig. 10.1) (Schoen and Brown 2001; Meyer et al. 2012). In essence, the crop genebank mission is to maintain "wildness" of the stored germplasm so that genebank users have full responsibility for domestication efforts.

Fig. 10.1 Seed of wild progenitors (left) and crops (right) showing domestication traits of lighter color and larger seeds. Top to bottom are *Pistacia*, *Coffea*, *Glycine*, *Hordeum*, *Zizania*, and *Sorghum*. Photo taken by LM Hill

Table 10.1 Requirements to ensure scientific collections are fit-for-purpose in studies of biological diversity

1	Maintain samples that are structurally intact (i.e., lack signs of physical damage) and are genetically representative of the source material (i.e., the conservation target)
2	Ensure samples are accompanied by data that describe the sample and the population from which it came
3	Authenticate data using accepted calibrations and standardized measurements
4	Allow access to samples and data
5[a]	Keep samples healthy and able to be regenerated

[a] A special requirement for germplasm banks

The purpose of this chapter is to describe some of the challenges one might experience when capturing and maintaining diversity inherent in collections of CWR – whether originating in North America or elsewhere. While we appreciate that plant (and animal) genebanks are often rationalized in an ethical context of conserving natural diversity (e.g., Soulé 1991; Guerrant et al. 2004, 2014), our premise in this chapter is that all the goals for genebanks – economic sustainability, environmental services, ethical considerations, opportunity for new applications, and aesthetic potential – are realized through scientific approaches. Hence, we view plant genebanks as scientific collections and that the scientists who use these collections need access to well-characterized, high-quality materials that are quality-assured so that the genebanking experience doesn't affect experimental outcomes (ISBER 2012; Walters et al. 2008; Guerrant et al. 2004, 2014). Overall requirements to gather and document materials that are fit-for-purpose for studies of biological diversity are summarized in Table 10.1.

Elements in Table 10.1 interact to contribute to the success (or failure) of genebanks in delivering samples of interest to users. Our focus in this chapter is element one and its interaction with element five. Providing viable samples (i.e., germplasm) differentiates a genebank from other types of plant collections such as DNA banks or herbaria. Arguably, ensuring viability while preventing genetic change is one of the greatest challenges facing genebanks today. Tools to validate how well a sample represents the source population and how it is maintained through time in the genebank are rapidly developing (Kilian and Graner 2012).

Plant genebanks can serve as an important tool for conservation. Ex situ conservation, made possible by genebanks, can complement in situ strategies that operate at habitat and landscape levels (Soulé 1991). Safely preserved at locations protected from social pressures or environmental disasters, genebanks can amass significant genetic diversity from a great range of taxa in a relatively small volume. Numerous land managers and conservation groups within the USA incorporate seed banking to forestall attrition of plant genetic diversity and ensure excellent sources of germplasm for land restoration (Guerrant et al. 2004; Hay and Probert 2013; Haidet and Olwell 2015; PCA 2015). Collections of plants of conservation concern (sensu USFS https://www.fs.usda.gov/Internet/FSE_DOCUMENTS/stelprd3848211.pdf (visited October 3, 2017)) exist already. These can be especially valuable as a source of agronomic traits (Khoury et al. 2013). For example, the highly endangered plant

Zizania texana Hitchc., which grows in a 7 km stretch of the San Marcos River near San Antonio, TX, has a desired perennial growth habit and produces seeds with exceptionally high lysine (Kahler et al. 2014).

Germplasm may be maintained in genebanks as samples actively growing, under field greenhouse or tissue culture conditions, or as alive-but-not-growing samples maintained by highly controlled conditions. Collections of the former are often referred to as living, and the latter can be referred to as stored, suspended (for suspended animation), or preserved. Often stored germplasm and seed banks are synonymous, although technologies have rapidly advanced to preserve many other germplasm forms in addition to seeds (see next section). Living collections allow curators to observe traits and regenerate samples, but they also increase the risk of losing samples to inclement weather, pests, pathogens, social unrest, and old age. Genetic erosion through drift, inadvertent selection, or introgression with neighboring related plants can also occur while growing or regenerating a sample. And, regeneration is especially expensive in terms of land and labor for large plants that may take years to sexually mature. Risks to field collections can be partially mitigated by maintaining plants in vitro, but labor and suitable space to maintain these collections can be cost-prohibitive (Pence 2011). Preserved collections are less expensive and more space efficient, carry lower risk from natural or anthropogenic disasters, and make genetic resources readily available regardless of season, year, or location (Li and Pritchard 2009; Volk et al. 2009; Pence 2011). Moreover, risks of genetic erosion during regeneration are mitigated when storage conditions are exceptional and maintain high viability with no mortality over extended periods (Richards et al. 2010; Walters et al. 2015a).

Plant genebanks are proliferating worldwide, and currently, about 1750 exist to serve agriculture, conservation, and studies of ecology, evolution, and diversity (Hay and Probert 2013; FAO 2014). These germplasm collections focus on a wide array of plant genetic resources and usually invoke a combination of living and stored approaches to maintain and evaluate samples. Major questions challenging genebank operations include forms of germplasm that can be preserved, propagated, and utilized, indicators for when a collection is "complete," life expectancy during storage, and assessment of genetic quality and potential uses of genebanked samples.

10.2 The "Conservation Target," Germplasm that Is "Fit-For-Purpose," and Genebank Management Plan

Germplasm samples must align with genebank mission, which is defined by the genebank's specific objectives or rationale. For example, the purpose of the USDA National Plant Germplasm System (NPGS) is to provide diversity that benefits research and education about agriculture. For this reason, NPGS collections focus on the subset of the world's approximately 300,000 plant species that have economic potential. Currently the NPGS collection contains only about 16,000 species, but this is represented by nearly 600,000 accessions (an accession is a sample with unique identifying information, such as taxon, location, and harvest details (this information is also called passport data)) – one of the world's largest plant germplasm collections globally (https://npgsweb.ars-grin.gov/gringlobal/query/summary.aspx visited October 3, 2017). NPGS accessions are roughly divided into named cultivars (50%), genetic stocks (20%), and wild relatives or landraces of crops (30%). About 250,000 accessions are distributed each year to users. This strongly suggests that interest in genebank collections among the scientific community resides in questions at the sub-taxonomic level.

Twenty five years ago, conservation targets for genebanks hovered at the taxonomic level (Soulé 1991). For example, botanical gardens used the living genebank strategy and broadly collected species but just had a few exemplars for each. This strategy provides support for phylogenetic distinctions but is unlikely to reveal variation within a species (Marshall and Brown 1975; Hokanson et al. 1998; Lawrence et al. 1995). Proliferation of genebanks that take advantage of advancing storage technologies and data management offer the opportunity for collections to explore a finer scale of genetic variation (Charlesworth et al. 2001; Lockwood et al. 2007a, b; Franks et al. 2008; Walters et al. 2008; Engelmann 2011). Conservation targets at these finer scales include populations, ecotypes, families (e.g., maternal lines), and individuals with exceptional characteristics, traits, or even particular gene expression patterns (Khoury et al. (2015). At the writing of this chapter, we feel that the conservation target(s) for CWR is/are mostly undefined for most crop collections. Conservation targets might range from samples that provide phylogenetic representation to samples that confer particular traits or ecotypes (e.g., drought tolerance). In many instances, collections of CWR are sought to provide a general representation of population diversity of the species, as a contingency against outbreak of disease or pests. When the conservation target is defined below the taxonomic level, stringency for maintaining genetic identity of the sample tightens (Table 10.1, element 1) and requires metrics to demonstrate the proficiency by which a genebank delivers samples that reflect the finer-scaled conservation target (Van de Wouw et al. 2010).

10.2.1 Germplasm

What part of the plant should be sampled for genebanking purposes? For DNA analyses, a fresh leaf or other non-senescent tissue is usually sufficient (Walters and Hanner 2006). However, for genebanks with the additional responsibility of providing live material (Table 10.1, element 5), the choice of propagule is a critical decision (Volk and Walters 2004). Within the genebank, ability to provide samples of high structural integrity that faithfully represent the conservation target usually rests on whether the material can be stored and easily distributed. Costs of processing and storage should figure significantly into the genebank's business model to determine the volume of material that can be managed effectively. Additional criteria for the collector might be timing of the collection trip in relation to plant phenology, remoteness of populations, permitting allowances, amenability to harvest, impact to the population and site, and potential for opportunistic collection of other species. Users may have additional preferences for the ease and required time for growing or propagating the material as well as the immediate availability of germplasm. Luckily, many plants are fairly plastic in their reproductive behavior and offer numerous propagule types to meet a range of requirements, preferences, and constraints (Table 10.2). Genebanks frequently distinguish between propagules that are sexually-derived (i.e., seeds and pollen) and those that arise from vegetative cuttings (i.e., clonally propagated). In agriculture, this distinction usually occurs because the conservation target is a specific genotype and the plant is highly heterozygous and outcrossing, for example, fruit crops (Volk and Walters 2004). Clonal propagation may be necessary for plants of conservation concern if there is reproductive failure in the wild (e.g., inbreeding, no pollinators) or if population sizes are inviable (Pence 2013). Since the conservation target of CWR is usually at either the population or gene level, stringent control of the genotype may be unnecessary and may actually impede broader representation of diversity within the population or incorporation of useful genes into a cultivar (Volk and Walters 2004).

Seeds are the most commonly used propagule for plant genebanks. Usually compact, plentiful, storable, growable, and representative of maternal and pollen-donor lines, seeds might just be the ideal medium for plant genebanking. Indeed, over 95% of the USDA National Plant Germplasm System collection uses seeds as the propagule form of choice. Seed-related traits such as fertility, fecundity, uniformity, germination speed, harvesting ability, and longevity – traits that facilitate genebanking – reflect traits selected during domestication (Meyer et al. 2012) (Fig. 10.1). We should expect disparities in these seed traits between untamed wild progenitors and their derivative modern cultivars. Consequently, we should also expect contrasting response to genebanking from domesticated and wild-collected germplasm. Moreover, we can expect wild-collected germplasm to be more prone to genetic erosion arising from the highly artificial conditions implicit within a genebank. Anecdotal accounts of greater difficulties genebanking seeds from the wild are increasing (Hay and Probert 2013; Walters 2015a). Despite these challenges, conservation groups and land managers have demonstrated the feasibility of genebanking wild seeds and the utility of this germplasm in restoration work (Maschinski and Haskins 2012; Guerrant et al. 2004; Haidet and Olwell 2015). As collaborators in these efforts, we have gained experience working with the seeds of truly wild species and can describe some of the pitfalls we've encountered that reduced the efficiency and accountability of genebanking efforts. These are not insurmountable problems; they simply indicate the need for adjustments in procedures, expectations, and anticipated costs for properly genebanking populations from natural populations compared to established methods using crop seeds.

Depending on several factors, seeds may be a less preferred germplasm form for sampling CWR in natural populations. Some plant species produce seeds that are less suited for genebanking because viability is lost quickly when standard genebanking conditions (sensu FAO 2014) are used. Seeds exhibiting low survival

Table 10.2 Some common propagules used in plant genebanks

Propagule	Advantages	Disadvantages	Exceptions
Seeds: Conservation target at population and/gene level	Compact High fecundity of some plants make it possible to collect many individuals Highly developed, low-cost, storage technology for orthodox seeds Efficient for propagation and regeneration and distribution Represents progeny of extant population (can capture many genotypes and many genes) May present barrier to some diseases Demonstrated ability to efficiently capture diversity	Heterogeneous traits in wild populations multiple harvest times needed, and timing can be unpredictable Asynchronous germination can lead to poor stand establishment and drift Long time to sexual maturity in perennials Potentially unknown pollen source Mating systems may preclude maintaining desired maternal traits	Non-orthodox seeds require cryogenic storage Possible low seed production in wild due to reproductive failure (endangered species), drought, late frost, non-mast year, herbivory
Pollen: Conservation target at gene level	Very compact Available for immediate use in breeding programs Available during flowering Amenable to storage Captures diverse genes Maybe the fastest, least labor-intensive way to achieve some form of back-up	A gamete, not an individual Ephemeral Difficult to harvest Must make crosses to regenerate populations Must be genebanked immediately after collection (short processing timeline)	
Shoot tips: Conservation target at individual level	Compact Captures specific genotype, OK as an exemplar of species Amenable to in vitro culture Preservation technologies rapidly developing Clonal propagation reduces concern about genetic drift	Requires large amounts of quality source materials at correct phenological stage Unexplained variation in response to growth medium among genotypes Processing and growth are labor intensive Many individuals needed to capture diversity of a heterogeneous population	

(continued)

Table 10.2 (continued)

Propagule	Advantages	Disadvantages	Exceptions
Dormant buds or overwintering vegetative structures: Conservation target at individual level	Compact Captures specific genotype, OK as an exemplar of species Does not require in vitro culture (less labor than shoot tips) Preservation technologies are advancing Clonal propagation reduces concern about genetic drift	Plants must be winter-adapted and in acclimated state Recovered by grafting Many individuals needed to capture diversity of a heterogeneous population	Variable responses within and among species result from complex bud structures
Somatic embryos and cell cultures: Conservation target at individual level	Compact Captures specific genotype; May be more amenable to preservation than non-orthodox seed Can generate huge numbers of individuals	Successful propagation is highly genotype-specific, tends to narrow captured diversity High risk of somaclonal variation Labor intensive for establishing and processing	

See also Havens et al. (2004) for complementary information

under standard genebanking conditions are nominally classified as “recalcitrant” or “intermediate” (and collectively as “non-orthodox”) compared to counterparts that are considered “orthodox” (Walters 2015b; RBG 2017). In interspecies hybridization zones, cuttings may provide more certain taxonomic identification than progeny from uncertain pollen sources. At sites where there are few individuals or low fecundity, cuttings may provide a means to collect germplasm with lower potential impact to the natural population; these can then be grown-out in field collections to facilitate characterization and regeneration through seeds. Pollen is under-appreciated as a germplasm form in plants, which contrasts with animal genebanks in which semen, the counterpart to pollen, is the most commonly used germplasm form (Mazur et al. 2008). Pollen might be an effective alternative germplasm form that can capture genes of interest and deliver them to a breeding population when seeds are unavailable or have poor storage characteristics or when maintaining cuttings is cost-prohibitive. For example, pollen from oak trees is desiccation tolerant, while oak seeds tend to be recalcitrant (Franchi et al. 2011). Pollen is storable (Hoekstra 1995; Volk 2011), but it lacks the longevity traits exhibited in seeds of the most common agronomic species (Dafni and Firmage 2000). The requirement for rapid processing of pollen samples makes it a less-ideal germplasm form when collecting from remote natural populations.

Quality of seed set and phenology are also important factors; a plant collector should sample seeds in the fruiting period, vegetative tissues when plants show active growth flushes or cold-adapted twigs are available, and pollen if plants are flowering. Alternative germplasm forms, such as pollen or cuttings, may augment genetic diversity lost by high mortality during banking of non-orthodox seeds.

10.2.2 Sampling Strategies and Management Plans.

In addition to the type of germplasm collected, the conservation target also defines the sampling strategy (Guerrant et al. 2014; Hoban et al. Chap. 8, this volume) as well as the genebank management plan. For germplasm banks, management plans must be suitable to deliver viable germplasm (Table 10.1, element 5); however management plans can vary depending on the conservation target. When the conservation target is an exemplar of phylogenetic representation, sampling probably occurred at one or a few convenient locations, and there probably wasn't great effort expended to get an accurate genetic representation of the species or particular populations. In this case, management at the genebank should complement the sampling effort to ensure sufficient viability for representatives of the taxon. Conservation targets for agricultural-based genebanks are usually at the sub-taxonomic level, for example, diversity is sought for a specific trait (e.g., aluminum tolerance, salt tolerance, and disease resistance) or for broad population representation needed for contingencies in the future. For conservation targets at trait or population levels, sampling usually occurred across diverse locations, and the sites and number of sites were selected carefully to maximize the sought diversity with fewest possible samples (so as to not overwhelm genebanking operations). Stringent genebank management plans are needed to ensure that the sample remains genetically representative of the source population. These are discussed further in Sect. 10.4 (Standards and Best Practices).

An important question arises about collection completeness, "completeness" being defined as how well the samples in the collection represent the diversity within the conservation target. There are few specific metrics to determine completeness (though see Hoban et al. Chap. 8, this volume for fuller discussion). It is important to note that metrics will differ among collections that are focused on different conservation targets such as a species representative, a specific trait or a contingency collection. International policy and legislation (e.g., International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA-FAO 2001), the Convention on Biological Diversity Strategic Plan (SCBD 2010), and the Global Strategy for Plant Conservation (SCBD 2014)) encourage strategies for ex situ conservation of CWR but provide few recommendations for the conservation target or metrics for effectiveness of sampling or management plans. Using species distribution models along with validating genetic data (Hoban et al. Chap. 8, this volume) may provide collectors more sophisticated tools to locate and monitor genetic variation and estimate uncertainty about collection "completeness."

10.3 Genebanking Wild-Collected Germplasm

Maintaining germplasm collections within allotted resources is a top priority for most genebanks. As mentioned earlier, living genebanks are limited in the amount of genetic diversity they can represent, and living germplasm is more vulnerable to stressful conditions. Preserved germplasm is maintained under highly controlled conditions. There is an expectation that it will remain viable into the future, but that duration is usually poorly defined. Often genebanking duration is defined as short-, medium-, or long-term, which most commonly define the storage conditions (ambient, refrigerated, and freezer, respectively) (FAO 2014), rather than the needed longevity, such as over a breeder's career, until an imminent restoration project is implemented, or forever. At NLGRP, we target a 100-year lifespan for most of our seed accessions (Walters et al. 2004).

The apparent stasis imposed by preserving germplasm often lures the naïve into a perception of simplicity; however the complexity of the effort is revealed by considering the timescale in which genebanks operate – usually decades. It is not generally appreciated that the impacts of seemingly minor deviations today won't be evident until sometime in the future; hence, an unsuspecting genebank manager may inherit a "ticking time-bomb." Genebank failures can go unexplained without standardized methods or stringent documentation. Herein lies a paradox: how do genebanks use standardized treatments for diverse materials and not encounter highly variable responses? And, do the variable responses impact how well the sample can represent the conservation target? A better understanding of time-scales is required to address these questions.

10.3.1 Stopping the Clock: A Primer in Preservation Technologies.

Understanding how to stabilize biological materials and predict the effects of time is a highly practical science needed for everyday problems. The food industry needs to provide expiration dates for product quality. Effectiveness of drugs and dosage response must consider the variable conditions that occur in household medicine cabinets. Plastic products, coverings, and packaging lose form and function over time. Everyone experiences the yellowing of old paper, the brittleness of aged rubber bands, and the failure of worn tape to hold documents together. Like all materials, the fundamental process for structural stability (i.e., preservation) is solidification (Menard 2008; Walters et al. 2010) – in the case of germplasm, this involves solidifying, or vitrifying, cytoplasm (i.e., forming a "glass") without too much disruption to the cell structure.

Most cytoplasm vitrifies at room temperature when samples dry to between 30% and 50% RH. If this level of drying can be accomplished without too much cell shrinkage, the cell survives; loss of more than 50% cell volume is considered lethal (Walters 2015b). During embryogenesis, food reserves (starch, protein, or lipid) are deposited into cells, displacing water which consumed as much as 80–90% of the cell volume in an immature embryo and 60–80% of cell volume in a mature recalcitrant (i.e., desiccation-sensitive) seed. The cell volume of a mature orthodox seed changes very little during desiccation (less than 30%), and we believe this explains their extreme tolerance to desiccation as well as subsequent longevity (Walters 2015b). The process of drying without dying makes desiccation tolerant organisms, including orthodox seeds, the original material engineers. This process is highly regulated during embryogenesis (Righetti et al. 2015), and incompletion or disruption of the established program, like any material, can have dire consequences to the functionality and stability of the end product – in this case, seed survival through time (Walters et al. 2010; Walters 2015b).

Once cells are in the glassy state (at temperatures below the glass transition temperature or Tg), they are relatively stable, meaning that change occurs, but over a much longer time scale than reactions occurring in fluid systems. In other words, preservation doesn't stop the clock, it just slows it down. In solids, such as vitrified cytoplasm, structure and mobility become two sides of the same coin. The "structure" is defined by how the compressed molecules impede movement of neighboring molecules. Pores formed during glass formation and molecules in the glass now shift to fill those pores and pack more efficiently. This rearrangement defines the "mobility" as well as the rate of change within the glass (Menard 2008). The movement brings molecules slowly into closer proximity, where they interact and oxidize; the material becomes brittle. The time scale is often experimentally intractable, which is one of the reasons why seed longevity is difficult to predict or measure. Water is a "plasticizer" of biological glasses, meaning it promotes larger pore space, hence greater mobility and faster aging. If seeds are not sufficiently dried, molecules in the cytoplasm move faster, causing more rapid deterioration. Anti-plasticizers (e.g., cryoprotectants) stabilize structure by a number of mechanisms. There is some speculation that anti-plasticizers of unknown identity accumulate in long-lived seeds (Walters 2015b).

A glass is stabilized by lowering the temperature. For orthodox seeds, which formed glasses during drying at ambient temperatures, molecular rearrangements to form lethal ice crystals at sub-zero (°C) temperatures are improbable, and so longevity increases progressively with lower temperature (to a point) (Walters, 2004). Most genebanks use freezers at −18 °C for conventional storage, because it is highly accessible technology, being easily achieved using a single-stage compressor. Freezer storage of plant germplasm began in the 1970s, and there was strong debate about its benefits until the early 2000s (Zheng et al. 1998; Walters 1998).

Cryogenic storage for plant germplasm became accepted in the mid-1980s and routine in the mid-1990s. Cryogenic storage may occur through a number of platforms, and the appropriate treatment and storage temperature for plant germplasm relies on the vitrification temperature, Tg. Technically, all storage below 0 °C is cryogenic; however, here, we consider it as storage below temperatures achieved by conventional freezers. Mechanical freezers with a dual-stage compressor cool to −80 °C and may be effective for germplasm with a relatively low Tg or unstable glass or when lipid transitions are important to survival. Most frequently, cryogenic storage is associated with the use of Dewar flasks or cryovats that are cooled by liquid nitrogen. Germplasm is either immersed into the fluid and stored at −196 °C or stored in the vapor above liquid nitrogen (between −150 and −190 °C, depending on distance from fluid surface and convection within the tank). A few status reports on longevity of cryopreserved germplasm are available (Towill et al. 2004; Walters et al. 2004; Volk et al. 2008; Ballesteros and Pence 2017; Pence et al. 2017).

Many plant propagules do not survive the desiccation stress required to form glasses at ambient temperatures (e.g., vegetative propagules and some non-orthodox seeds) (Table 10.2). Lowering the temperature of these non-vitrified systems poses high risk of lethal ice formation, which can only be avoided by forming a glass during the cooling process and maintaining it below Tg so that glasses don't melt and ice doesn't form during storage. Inhibiting ice formation at sub-zero (°C) temperatures, while maintaining cell viability, requires optimization of interacting treatments for moisture adjustments, additions of cryoprotectants and rapid cooling (Walters et al. 2013; Wesley-Smith et al. 2014).

10.3.2 Conservation Targets, Sample Quality, and Preservation Success

We preface this section by the infamous story of the Pará rubber tree, *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg., which produces a non-orthodox seed. Mostly told for political intrigue, the story describes how less than 1% of over 50,000 seeds survived to domesticate the species and initiate rubber industries in Malaysia and Singapore (Brockway 1979). Here, the conservation target for *H. brasiliensis* was primarily its rubber-producing trait, a species characteristic that can be captured by exemplars. Thus, loss of 99% of the collected seeds, as a result of poor shelf-life, was considered acceptable. We would likely find those losses unacceptable by today's standards because our conservation targets tend to be at finer scales: genetic diversity representative for the whole species or for key populations or individuals. The salient point, for the context of this chapter, is that preservation success is defined by whether loss of genetic diversity during genebanking is acceptable, which is largely dependent on the stated conservation target.

The *Hevea brasiliensis* story also illustrates that it is possible to genebank even when seeds are not orthodox. Usually a species producing non-orthodox seeds will be harvested as a cutting or fresh seed and immediately grown out to form a living collection. As described in the previous section, it is now possible to preserve non-orthodox seeds and other germplasm forms that do not survive cytoplasmic solidification at ambient temperatures. However, this effort requires exacting coordination between collector and curator.

An initiative to bank wild species requires background information on how propagules respond in storage. To that end, we cross-referenced prioritized CWR from the USA (Khoury et al. 2013) with information available from Royal Botanic Kew's

Seed Information Database (SID) (RBG 2017) (Table 10.3). A limited number of US species were included in the SID, emphasizing the lack of information available for storage behavior of germplasm from CWR native to the USA. Extrapolating from the genus level, we believe that 75% of the 179 genera from the Inventory will produce orthodox seeds and that at least 8% will not produce orthodox seeds. High variability in seed storage response is exhibited in about 8% of congeners, and no records are given for 8% of the genera.

Even orthodox seeds present challenges for storage, especially with conservation targets at the population level, which is where most agronomic- and conservation-based collections are poised. The increasing number of anecdotal accounts that seeds collected from the wild are harder to store are not surprising (Hay and Probert 2013; Walters 2015a; Balleseros and Pence 2017). We know that embryo development is critical to longevity, and metabolic pathways expressed during embryogenesis are keys (Righetti et al. 2015; Walters 2015b). Seed quality is dependent on processes that are uncontrolled in the wild during the growing season, such as moisture availability, nutrition, competition, and pathogens, and it will decline if developmental programs are not completed (Probert et al. 2007) or extended toward germination (Tarquis and Bradford 1992). Seed quality is also under genetic control (Clerkx et al. 2004; Schwember and Bradford 2010; Nagel et al. 2011; Righetti et al. 2015), with ecotypes within a species having contrasting storage behavior (Tweddle et al. 2003; Clerkx et al. 2004; Daws et al. 2004; Walters et al. 2005; Probert et al. 2009; Kochanek et al. 2009; Mondoni et al. 2014). Phenology, fecundity, carbon partitioning, composition, seed coverings, resistance to pests, and drought tolerance are all inherited traits that affect seed longevity. These traits are more uniform in domesticated plants but vary considerably in seeds from natural populations; hence, an accession of seeds collected from the wild will be heterogeneous, and this will result in differences on how individual seeds within the sample respond to genebanking conditions.

Genebanking wild-collected seeds carries inherent risks for genetic erosion: when the shorter-lived seeds in an accession die, seed traits will tend toward greater uniformity, and when seeds are regenerated, germination, flowering, and maturity are likely to become more synchronized. Unless extraordinary measures prevent these tendencies, genebanking seeds collected from the wild can be an exercise in domestication as a result of inadvertent selection of traits controlling preservability, growth habit, morphology/physiology, and reproductive capacity (e.g., Burton and Burton 2002; Gilligan and Frankham 2003; Harding 2004; Aubry et al. 2005; Falk et al. 2006). Even though sample quality may remain high during genebanking, there may be an attrition of sought-after genetic diversity and an increasing tendency for genetic representation of the sample to veer away from the source population (Table 10.1, element 1).

Table 10.3 Probable seed storage behavior for taxa native to the USA that are congeneric to domesticated species

Genus with a 1A, 1B, or 2 priority ranking[a]	# accessions in NPGS for genus[b]	# priority taxa for priority collection in the USA[a]	Predicted response to storage[c]	# of species within genus with reported constants for Seed Viability model[d]
Abutilon Mill.	0	8	O	0
Acer L.	0	6	O to R	1
Actaea L.	47	1	O	0
Aegilops L.	0	5	O	0
Agave L.	20	4	O	0
Agropyron Gaertn.	0	2	No data	
Agrostis L.	0	15	O	0
Allium L.	0	47	O	1
Alopecurus L.	0	4	O	0
Amaranthus L.	3353	40	O	0
Andropogon L.	0	13	O	0
Annona L.	48	1	O to U	
Apios Fabr.	0	1	No data	
Apium L.	0	1	O	0
Arbutus L.	0	3	O	0
Armoracia G. Gaertn.	0	1	O	0
Aronia Medik.	0	3	No data	
Arrhenatherum P. Beauv.	0	2	O	0
Artemisia L.	0	50	O	0
Artocarpus J.R. Forst. and G. Forst.	0	1	R	
Asimina Adans.	1024	9	U	
Asparagus L.	0	3	O	0
Atriplex L.	0	37	O	0
Avena L.	0	3	O	0
Bassia All.	0	1	O	0
Beta L.	0	4	O	1
Boehmeria Jacq.	0	1	O	0
Brassica L.	0	5	O	2
Bromus L.	0	35	O	0
Camelina Crantz.	0	1	O	0
Canavalia Adans.	0	6	O	0
Capparis L.	0	2	O to R	
Capsicum L.	5084	2	O	0
Carica L.	53	1	I	
Carthamus L.	0	1	O	0
Carya Nutt.	4078	13	U	
Castanea Mill.	15	5	R	

(continued)

Table 10.3 (continued)

Genus with a 1A, 1B, or 2 priority ranking[a]	# accessions in NPGS for genus[b]	# priority taxa for priority collection in the USA[a]	Predicted response to storage[c]	# of species within genus with reported constants for Seed Viability model[d]
Chenopodium L.	386	51	O	0
Chrysanthemum L.	0	1	O	0
Chrysophyllum L.	0	2	R	
Cinnamomum Schaeff.	0	1	R	
Cochlearia L.	0	1	O	0
Cocos L.	0	1	R	
Coix L.	0	1	O	0
Colocasia Schott.	0	1	U	
Corchorus L.	0	2	O	0
Coreopsis L.	0	8	O	0
Corylus L.	803	3	I	
Crataegus L.	0	70	O	0
Crotalaria L.	0	6	O	0
Croton L.	0	15	O	0
Cucumis L.	0	4	O	1
Cucurbita L.	3392	8	O	1
Cuphea P. Browne	0	5	O to I	
Cynara L.	0	3	O	0
Cyperus L.	0	48	O	0
Dactylis L.	0	1	O	0
Daucus L.	1578	2	O	0
Digitaria Haller	0	20	O	0
Dioscorea L.	0	3	O	0
Diospyros L.	0	7	O to R	
Diplotaxis DC.	0	2	O	0
Echinacea Moench.	0	13	O	0
Echinochloa P. Beauv.	0	15	O	0
Elymus L.	0	43	O	0
Eragrostis Wolf	0	27	O	0
Eruca Mill.	0	2	O	0
Eugenia L.	0	3	R	
Fagus L.	0	2	O to I	
Festuca L.	0	36	O	0
Ficus L.	0	4	O	0
Foeniculum Mill.	0	1	O	0
Fragaria L.	1907	21	O	0
Gaylussacia Kunth.	0	8	O	0
Glycyrrhiza L.	0	1	O	0

(continued)

Table 10.3 (continued)

Genus with a 1A, 1B, or 2 priority ranking[a]	# accessions in NPGS for genus[b]	# priority taxa for priority collection in the USA[a]	Predicted response to storage[c]	# of species within genus with reported constants for Seed Viability model[d]
Gossypium L.	10,582	3	O	1
Hedysarum L.	0	7	O	0
Helianthus L.	5158	72	O	1
Hibiscus L.	0	18	O	0
Hordeum L.	0	18	O	1
Humulus L.	626	6	O	0
Hydrastis J. Ellis	0	1	No data	
Hypericum L.	0	1	O	0
Ilex L.	0	21	U	
Illicium L.	0	1	R	
Ipomoea L.	1251	40	O	0
Jatropha L.	0	4	O	0
Juglans L.	702	9	U	
Lactuca L.	2943	11	O	1
Lathyrus L.	0	31	O	0
Lepidium L.	0	37	O	0
Lespedeza Michx.	0	11	O	0
Leymus Hochst.	0	17	O	0
Licania Aubl.	0	1	No data	
Lilium L.	0	5	O	0
Limnanthes R. Br.	82	1	O	0
Linum L.	0	21	O	0
Lolium L.	0	3	O	0
Lotus L.	0	77	O	0
Lupinus L.	0	95	O	0
Malus Mill.	6203	4	O	1
Manihot Mill.	21	4	O	1
Manilkara Adans.	55	1	I to R	
Medicago L.	0	10	O	0
Melilotus Mill.	0	3	O	0
Mentha L.	0	4	O	0
Mespilus L.	0	1	No data	
Morus L.	0	2	O	0
Nasturtium W.T. Aiton	0	4	O	0
Nicotiana L.	2342	9	O	0
Olea L.	0	1	O	0
Oplopanax (Torr. and A.Gray) Miq.	0	1	O	0
Opuntia Mill.	286	31	O	0

(continued)

Table 10.3 (continued)

Genus with a 1A, 1B, or 2 priority ranking[a]	# accessions in NPGS for genus[b]	# priority taxa for priority collection in the USA[a]	Predicted response to storage[c]	# of species within genus with reported constants for Seed Viability model[d]
Oxalis L.	0	8	O	0
Pachyrhizus Rich. Ed DC	11	0	O	0
Panax L.	0	1	No data	
Panicum L.	1731	37	O	0
Papaver L.	0	14	O	0
Parthenium L.	151	7	O	0
Paspalum L.	0	42	O	0
Passiflora L.	0	13	O to I	
Pastinaca L.	0	1	O	0
Pennisetum Rich.	0	10	O	1
Penstemon Schmidel	0	39	O	0
Persea Mill.	173	3	R	
Phalaris L.	0	6	O	0
Phaseolus L.	17,856	17	O	1
Phleum L.	0	2	O	0
Phlox L.	479	9	O	0
Physalis L.	0	13	O	0
Physaria (Nutt. ex Torr. and A. Gray) A. Gay	237	4	O	0
Pinus L.	0	4	O	0
Piper L.	0	1	O to I	
Pistacia L.	356	1	O	0
Poa L.	0	42	O	0
Portulaca L.	0	10	O	0
Pouteria Aubl.	85	0	R	
Prosopis L.	0	9	O	0
Prunus L.	2970	30	O	0
Psathyrostachys Nevski	0	2	No data	
Pseudoroegneria (Nevski) Á. Löve	0	1	No data	
Psidium L.	67	2	O	0
Pueraria DC.	0	3	O	0
Pyrus L.	0	1	O	0
Raphanus L.	0	1	O	0
Rhododendron L.	0	30	O	0
Ribes L.	1273	65	O	0
Rorippa Scop.	0	9	O to U	

(continued)

Table 10.3 (continued)

Genus with a 1A, 1B, or 2 priority ranking[a]	# accessions in NPGS for genus[b]	# priority taxa for priority collection in the USA[a]	Predicted response to storage[c]	# of species within genus with reported constants for Seed Viability model[d]
Rosa L.	0	27	O	0
Rubus L.	2109	67	O	0
Rudbeckia L.	0	11	O	0
Ruellia L.	0	2	O	0
Rumex L.	0	19	O	0
Saccharum L.	0	9	O	0
Salsola L.	0	4	O	0
Sambucus L.	0	11	O	0
Satureja L.	0	1	O	0
Scorzonera L.	0	1	O	0
Setaria P. Beauv.	1081	27	O	0
Simmondsia Nutt.	324	1	O	0
Solanum L.	18,016	39	O	0
Sorbus L.	0	11	O	0
Sorghum Moench.	0	4	O	1
Stillingia Garden	0	2	No data	
Syzygium R.Br. ex Gaertn.	0	2	R	
Theobroma L.	271	0	R	
Thinopyrum Á. Löve	0	2	No data	
Thlaspi L.	0	1	O	0
Tragopogon L.	0	4	O	0
Triadica Lour.	0	1	O to R	
Trifolium L.	0	96	O	0
Tripsacum L.	294	4	No data	
Vaccinium L.	1786	39	O	0
Vanilla Mill.	0	2	No data	
Vernicia Lour.	0	1	No data	
Vicia L.	0	14	O	0
Vigna Savi	0	2	O	2
Vitis L.	5028	29	O	0
Zizania L.	0	6	I	

Genera are from supplemental material supplied by Khoury et al. (2013). Information about seed responses to storage are from congeners listed in Kew's SID (http://data.kew.org/sid/sidsearch.html accessed 14 Feb 2017). Information on constants for the Viability Equation model come from a different page on the SID website (http://data.kew.org/sid/viability/ accessed October 3, 2017) and represent the number of species within the listed genus with reported constants (no data were available for specific taxa listed the Inventory)

[a] Definitions of priority rankings for collection (1A, 1B, and 2) were taken from Khoury et al. 2013, and associated taxa were retrieved from the Inventory provided at http://www.ars-grin.gov/misc/tax/ (accessed 6 Feb 2017)

(continued)

Table 10.3 (continued)

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[b] Presence of accessions for genera within NPGS collections were retrieved from GRIN (site) (Courtesy of C.K. Khoury)

[c] Seed storage behavior was taken from Kew's SID and reflect behaviors listed for the majority of congeners with reported data (no data were available for specific taxa listed in the Inventory) (http://data.kew.org/sid/sidsearch.html accessed October 3, 2017), O, orthodox; I, intermediate; R, recalcitrant; U, unclear

[d] Congeners with Viability Equation information come from a different page on the SID website (http://data.kew.org/sid/viability/ accessed October 3, 2017) (no data were available for specific taxa listed the Inventory). Zero indicates no information listed for that genus; blanks indicate seeds with probable non-orthodox storage behavior

10.3.3 Predicting Longevity and Detecting Aging

The intention to use genebanking to conserve genetic diversity extant in the wild underpins genebanking practices designed to prolong seed shelf-life. Storage treatments, viability monitoring frequencies, and regeneration are all based on assumptions about longevity. However, longevity varies tremendously among seed lots within a species due to uncontrolled and unknown factors of seed quality. Therefore, the actual longevity of a seed lot is only known after-the-fact. Genebanks need reliable assessments of longevity before and during storage.

Longevity might be predicted using an empirical model that is parameterized by constants for moisture and temperature effects for different species. The Seed Viability Equations (Ellis and Roberts 1980) [http://data.kew.org/sid/viability/ (visited October 3, 2017)] provide "ball-park" estimates of survival with time when conditions are not-too-cold or not-too-dry; this model becomes unreliable beyond the limits of inference of the data used to parameterize it (Walters 1998; Walters et al. 2004). Since freezer storage is a relatively new practice (it was first used routinely at NLGRP in 1978), there are few data sets that actually demonstrate longevity in the freezer, let alone predict it reliably.

Models such as the Viability Equations allow us to "standardize" different laboratory experimental conditions, so diverse species can be ranked for longevity in a similar context (Hay et al. 2003). Not surprisingly, information for CWR species from the USA (Khoury et al. 2013) are not available. The information might be gleaned from behavior of congeners, but only 15 of the 135 genera believed to be orthodox had species coefficients listed in the SID (Table 10.3). More extensive comparisons of seed longevity within the genebank, and estimates of within-species variation for some CWR, come from early genebank results of seeds stored initially at 5 °C (Walters et al. 2005; Nagel and Börner 2010). Additional insights come from seed aging experiments conducted at warmer temperatures under high humidity challenges (Probert et al. 2009) or drier conditions (e.g., Fig. 10.2). There is general agreement that seeds from Apiaceae tend to be short-lived and seeds from Chenopodiaceae tend to be long-lived. Seeds from Asteraceae and Poaceae exhibit a wide range of longevities. In the future, we hope to adjust species-level information with data about habitat, location, and weather data associated with the sample.

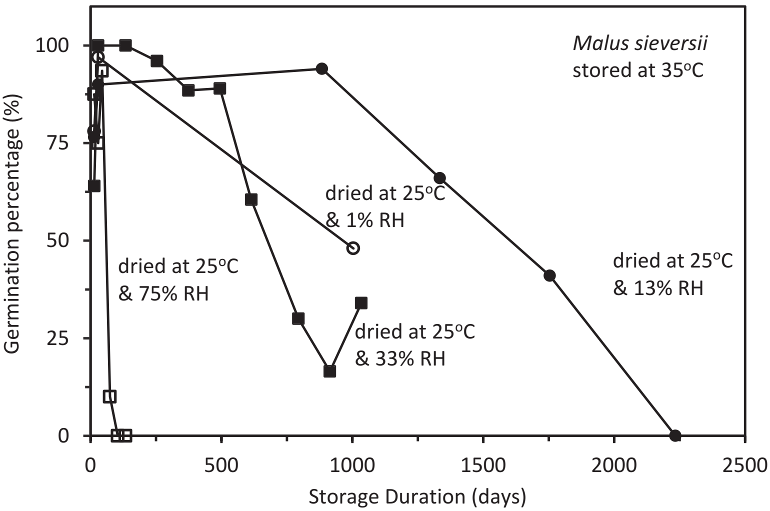

Fig. 10.2 Survival of a seed sample of *Malus sieversii*, progenitor species of domesticated apple. Seed moisture was adjusted at 25 °C at indicated relative humidity for a 3-week period; seeds were then sealed in foil laminate packages and placed at 35 °C for indicated time and then germinated. The increasing longevity with decreasing moisture treatment (75–13% RH) is indicative of orthodox behavior, and the faster aging at very low RH (1%) demonstrates a limited benefit of drying. This seed lot survived for about 3 years (+1000 days) at 35 °C. Extrapolating longevity to −15 °C using a general rule for temperature effects (doubling for every 10 °C; Q10 = 2), we can predict this seed lot would survive for about 100 years in a genebank (original data)

Wide variation of longevity within a species makes it difficult to accurately predict how a particular sample will behave in the genebank (e.g., Walters et al. 2005). These differences are accommodated in the Viability Equations using an initial quality factor, which is dependent on a highly accurate measure of initial germination as well as an assumed high correlation between initial germination and longevity. Despite model predictions, there is a poor correlation between initial germination and longevity (Walters et al. 2005; Nagel and Börner 2010; Ballesteros and Pence 2017), which we attribute to quality factors that have initial but not long-term effects (Mead and Gray 1999) and factors that have long-term but not initial effects (Hay and Probert 1995; Tarquis and Bradford 1992; Walters et al. 2004).

Monitoring viability is currently the genebank's only tool for assessing whether quality is maintained. For seeds, this involves a germination assay which, like the initial test, is a snapshot having little predictive power of future change. Statistical considerations related to sample size also influence how well change can be detected (Guerrant and Fiedler 2004; Richards et al. 2010). Accumulation of deaths in time, marked by changes in germination potential, is a poignant demonstration that responses of individuals *within* a sample vary during storage.

Genebanks must monitor seed viability, but without a priori information on longevity and germination, there is no guidance for monitoring interval or seed counts. When a priori knowledge is insufficient, frequent viability monitoring is recommended (FAO 2014); this demands extensive resources and can quickly deplete the sample, rendering the entire exercise useless. Better processing and storage conditions to prolong longevity, better methods to relate aging at higher temperatures to freezer conditions, and new tools to monitor nondestructively (Colville et al. 2012; Mira et al. 2016; Fleming et al. 2017) will aid the genebanking operation.

10.4 Standards and Best Practices

Standards or best practices communicate how state-of-art science should be implemented in order to maintain quality samples for a desired timeframe (ISBER 2012). They also communicate to future users how samples were treated (ISBER 2012). Intentions of standards are quality control, predictability, and metrics for how the genebanking experience affected the sample relative to its conservation target. This is particularly important if methods to accomplish bio-banking goals (sensu Table 10.1) are not yet established. Future users deserve to know the history of the sample and how protocols might affect the sample's usefulness to them. Therefore, best practices and standard operating procedures (SOPs) must convey information to users so that they can access whether the sample is fit for *their* purpose.

Standards and best practices for genebanking must align with the sampling strategy, which ultimately must align with the stated conservation target. In our opinion, the conservation target is often not sufficiently defined for samples collected from wild populations, and this can cause a mismatch between sampling and genebanking protocols. In the example of *Hevea brasiliensis,* ability to produce latex for rubber is expressed at the species level, which meant collectors needn't search for this useful trait among certain ecotypes or individuals. Collectors harvested an excessively large number of seeds because they had prior failures due to the difficult physiology – not because they wanted to capture genetic diversity (Brockway 1979). In other words, the primary conservation target was a few specimens of the species, and extraordinary measures to keep all 50,000 of the harvested seeds alive would have been initially unappreciated. Therefore, in this context, stringent management of the collection wasn't that necessary. Eventually, though, the new rubber industries learned that greater genetic diversity from higher seed survival would have been beneficial, and collections for genetic diversity within the species would have required more stringent genebanking protocols. Linking this analogy to the case of CWR within the USA, we need to know whether the agronomic traits we seek (e.g., Khoury et al. 2013) are expressed by all individuals of a species, by specific ecotypes, or by some rare individuals. We believe the answer to this question can help to define the conservation target as well as the technical investment required to maintain US collections.

Developing a set of agreed-upon standards or "best practices" (sensu ISBER 2012) for collecting and maintaining diversity invariably develops into discussions of practicalities, impossibilities, and available resources. Experienced genebanks know that working with wild-collected materials usually involves choosing among less-than-optimum options. Often, quality control of samples coming into the genebank is limited by the biology of the organism. Standards, such as FAO's Genebank Standards (FAO 2014), can guide genebanks established for agricultural purposes (domesticated plants and CWR), and modifications for wild plant species collected for conservation purposes have been suggested (Hay and Probert 2013). Currently genebanking standards for samples collected in the wild require initially high viability and frequent viability monitoring as well as management decisions to regenerate or recollect when viability degrades to 85% of initial viability (FAO 2014, Seed Conservation Standards for "MSB Partnership Collections" at http://www.kew.org/sites/default/files/MSBP%20Seed%20Conservation%20Standards_Final%2005-02-15.pdf (visited on October 3, 2017)). These stringent guidelines are to ensure the sample remains genetically representative of the wild population from which it is harvested (Table 10.1, element 1) (FAO 2014). If the conservation target is not specific to a population, is it necessary to follow these technically stringent standards? If the conservation target is specific to a population, does stringency of SOPs increase risks of genetic erosion by premature consumption of the sample through too much testing or too frequent or infrequent regeneration (Richards et al. 2010)?

FAO's standards for orthodox seeds (Chap. 4 in FAO 2014) serve as the foundation for many national and international seed banks around the globe, including the USA. These standards were developed about 7 years ago through consensus of a large group of experts having different opinions on the intent of standards and even the interpretation of existing storage data to guide standards. Remaining ambiguities and inaccuracies were place-marked for future research and understanding, and sufficient scientific knowledge has accumulated to call for an update, or at least modification, to meet the needs of seed banks. Improved methods are especially needed to address uncertainty associated with genebanking seeds when there is little knowledge about the species or contingencies when samples are heterogeneous and prone to genetic erosion. Current standards may also be too stringent for some conservation targets. Therefore, we take this opportunity to examine these standards for orthodox seeds and suggest areas for better alignment with conservation targets for germplasm collected from wild populations. To encourage conversation among genebanks, we have listed some standards we feel need adjustment and have provided alternative language (Table 10.4). Standards for non-orthodox seeds in Chap. 6 of the Genebanking Standards (FAO 2014) can also be modified to reflect rapidly developing technologies.

10.5 Sample Regeneration and the Nexus of Different Genebanking Strategies

Genebanking seeds collected from wild populations will likely impose genetic bottlenecks (Falk et al. 2006) that can be minimized by careful collection (Hoban and Schlarbaum 2014) and curation (Richards et al. 2010) as well as large enough sample sizes and treatments informed by the biology of the sample (Hay and Probert 2013). The extent and direction of genetic shifts can have large impact on the

Table 10.4 Best practices for acquisition and storage of orthodox seeds having agricultural utility

Standard	Explanation for difference from current FAO standard	Alternative best practice
Sampling from wild populations	The conservation target (e.g., a specimen, sample that is representative of the population, or single/multiple maternal line(s)) determines the sampling and maintenance strategies which, in turn, determines rigor of genebanking operations as well as costs	*Establish goals and comply with legal requirements* Clearly state conservation target Comply with all legal obligations for collection, transport, propagation, and distribution of samples. Obtain permission from landowners and any regulatory agencies to make seed collections and report permit or agreement numbers as well as handling and distribution restrictions in accession records. Acquire necessary permissions several months before the collecting season
	Sampling strategy should align with conservation target and population size. If Best Practice/Standard can't be met, consider redefining the conservation target to make it appropriate to sample from fewer individuals. For example, site has a single tree, the conservation target is more likely a maternal line rather than a whole population Sufficient seeds should be collected to accommodate curation, distribution, and regeneration of the accession. Sampling must not impact wild populations.	*Basic information about the population and how much to take* Distribute seed harvests among a representative proportion of maternal plants to meet conservation target goals. Standard number of plants for a population-based conservation target is about 50, and a greater number is needed if a desired trait or gene is believed to be rare in the population. Fewer maternal plants are acceptable if the conservation target is a specimen or exemplar of the species or a trait that is characteristic to the species To represent a natural population that is not of conservation concern, strive to collect at least 2500 seeds from a representative cross-section of plants, but do not collect more than 20% of available seeds. To represent a natural population that is of conservation concern, collect no more than 10% of the available seeds, and distribute sampling across at least half of the seed-bearing plants. If seed numbers are too low, consider various options: revision of conservation target, recollection at the site, or immediate regeneration of seeds to get requisite numbers for curation and distribution. Initial sample size should accommodate at least 20 average requests and 2 unusually large requests. Therefore, target ~1400 seeds (distribution) + 800 seeds (maintenance) + 250 seeds (regenerations × 3) + 50 seeds (archival) = 2500 seeds. Report the numbers of seed-bearing plants in the target population and proportion of plants sampled for seeds.

(continued)

Table 10.4 (continued)

Standard	Explanation for difference from current FAO standard	Alternative best practice
	Associated data should be in format compatible with national and international databases that compile information about biological collections. Standard should reflect information commonly sought by users.	*Associated data* Record data about the collection site using standard definitions and units. Direct measurements, rather than inferred information, especially for geospatial data, are always preferred. Using Darwin Core standards will ensure compatibility across institutions (Wieczorek et al. 2012, Endresen and Knüpffer 2012). Basic "passport" data should include categories for institution and sample identification, taxonomic classification, collector and collection date, general locality information, georeferenced latitude and longitude, and site ownership. Geospatial data should be measured directly and not inferred from locality information. Habitat fields should be consistent across institutions and include data on physical attributes of the site, such as elevation, slope, and aspect. Sampling data should include the number of individuals in the population at the site and number or proportion of individuals from which seeds were harvested (both reflect conservation target level for which a sample is appropriate/suitable).
	Seed maturity is a complex trait under genetic and environmental regulation. Sampling only mature seeds at a single date may impose a genetic bottleneck during sampling. Immature seeds age faster than fully mature seeds, and the faster aging may impose a genetic bottleneck during storage. If the conservation target is a representative sample of the population, it's important that the collection include seeds that matured at different times. If this requires inclusion of immature seeds (because only a single sampling date is possible), special treatment of the sample is needed to avoid bottlenecks during storage.	*Collect seeds that will survive banking* Collect mature seeds for greatest potential longevity. If goal is for sample to genetically represent the population, sample over the entire fruiting season, making multiple trips to the collection site. If only one harvest is possible (because the site is remote), sample the entire range of seed maturities representatively, and alert seed bank curator of need for expedited handling. A sample containing more than 10% immature should be targeted for cryogenic storage and needs to be processed quickly. For plants with very short fruiting season, obtain on-the-ground monitoring of seed maturity, and use collection procedures to capture only mature seeds. Report date(s) of seed collection; associated phenology data such as flowering time and range of maturity of seeds in the population are useful. Do not include seeds from other species in the sample. Immediately after harvest, put seeds under cool (12–15 °C for tropical seed, 5–10 °C for temperate seeds) aerobic conditions, out of direct light, and away from free water. Transmit samples to laboratory-controlled conditions within 1 week.

Processing before storage and storage conditions and safety back-up	Current standards don’t provide guidance on initial processing of seeds arriving at genebank.	*Receiving, cleaning, and weighing* Assign a unique, identifying number to each incoming sample and associated collection information, and record date of receipt. Clean sample to reduce bulk and apparent diseased materials, but do not aim for perfection if it takes time away from more vital activities. Keep sample cool and dry (RH < 50%) while cleaning. If debris within sample affects consistency among germination assays, the genebank may wish to prepare a subset for subsequent viability monitor tests. Record mass of seed using IS units. Convention is to record a single mass for 100 seeds; however, replicated measurement of individual seeds is more informative. The relative humidity associated with the seed at time of measurement is useful to assess dry mass. Record number of seeds in sample and an indication of whether number was a direct count or inference based on mass.
	Standards need to be revised to emphasize the point that postponing low temperature storage will negatively impact longevity at storage temperature. Thus, guidelines should emphasize drying times and anomalies in storage temperature. A requirement for “equilibration” to a specific moisture level suggests a certain exactness that is not supported by the literature. Moreover, some combinations of drying RH and drying temperature will result in costly over-drying of seeds when stored at −18 °C. For example, drying at 15% RH will always result in less than 15% RH if the sample is stored at lower temps. Re-equilibration will also always occur once the sample is moved to the storage temperature. Hence, there are many options for drying that will achieve the recommended moisture level, and choice of conditions should be a matter of ambient conditions, costs to dry, and workflow decisions.	*Drying samples to maximize longevity at storage temperature* Dry samples quickly to the appropriate moisture target (between 15 and 25% RH *at the storage temperature*). Drying should occur at less than 30C to avoid damaging the seed. Drying rate depends on drying temperature and should be complete within 2 weeks (dry at 25C) to 2 months (dry at 5C). To achieve moisture target at storage temperature, final RH of seed should not be less than 15%. Upper limit of drying RH is temperature dependent: 34% at 25C, 30% at 15C, and 25% at 5C. Samples should be dried closer to the lower RH range as the risk of storage temperature failure increases. Monitor RH and temperature of drying space continuously, and send automatic alarms when beyond range. Confirm target moisture level is achieved by spot checking the RH within storage containers *at the storage temperature* before completely sealing for 5% of seed samples. If slight adjustments are needed, add calculated quantities of an innocuous water-absorber, such as polished rice, at a pre-adjusted water content. Control RH of seed storage environment using water-impermeable packaging or humidity controls if seeds are not stored in water-impermeable packaging. WVTR (water vapor transmission rate) is the standard to measure water impermeability of packaging and should range from 0.003 to 0.006 g H_2O/m^2/day, depending on ambient RH (lower WTVR needed for ambient RH > 80% and higher WTVR is appropriate when ambient RH is <40%). Metal foil thicknesses of 25 and 10 μm meet the minimum standards for seed banks located in areas of high and low ambient RH, respectively, as long as seal is strong and foil layer is undamaged. Glass containers also have specified WTVR if gasket is frequently replaced. Monitor RH of storage environment surrounding seed packages and spot check RH within storage container in 1% of samples. Replace laminated bags and gaskets when seeds are monitor-tested or after about 30–40 years of freezer storage.

(continued)

Table 10.4 (continued)

Standard	Explanation for difference from current FAO standard	Alternative best practice
	Ideally, standards or best practices would be based on achieving specific longevities, such as 20 years for medium term and 100 years for long term. Because longevities can't be known in advance, standards and best practices are defined by storage temperature that is anticipated to provide the desired outcomes. Revised standards should accommodate possibilities of cryogenic storage, which has been accommodated here by allowing temperatures to be lower than the recommended range.	*Storage temperature to achieve required longevity* Store seeds maintained for use or distribution at 5 ± 3C (i.e., refrigerated or medium-term storage) or below. Store seeds maintained for safety duplicate samples using long-term storage conditions at −18 + 3C (i.e., freezer storage) or below. Record date that seeds were placed at storage temperature. Monitor storage temperature continuously, and have system wired to send automatic alarms when out of range. Prepare incident reports for anomalies documenting dates of malfunction and return to normal as well as temperature deviations. Keep samples away from the heating element of the defroster. Samples in long-term storage should experience temperature fluctuations very infrequently. It is best to remove just a subsample for viability monitoring and distribution while at temperature, so that the entire sample doesn't warm. If warming the entire sample is unavoidable, the sample retrieved from long-term storage should be held in a refrigerator and experience no more than 10 h of ambient room temperature per year. Check RH before resealing, and adjust as needed by slight drying or adding a moisture absorber, as described above.
		Use freezer conditions for archival and safety-duplication Most original samples should also be stored at −18 °C or below. Maintain more than 1500 seeds in safety-duplicate samples (FAO 2014). Wear proper protection, and use a buddy system or a "man-down" alarm system when entering freezer rooms. Follow all OSHA regulations.

Assessing seed quality	Of all seed banking procedures, best practices for initial viability tests and subsequent viability monitoring carry the greatest uncertainty and highest risks for unnecessarily depleting samples or not detecting deterioration in time for regeneration. It must be clear that the standards and best practices are for seeds that are known to be orthodox. If effects of drying and temperature are unknown, preliminary tests are required to ensure the appropriateness of these practices. Current standards attempt to define acceptable quality of seeds. But this can be tricky for wild-collected samples where quality can range considerably and valid methods to assess quality are usually unknown. Our aim here is to assess quality relative to existing knowledge about the species.	*Initial viability testing* Test seed viability soon after receipt at genebank using methods that will allow valid comparisons in subsequent monitor tests. Test the sample after it has been cleaned and weighed and no longer than 8 months after receipt. The earlier the viability test is conducted after harvest, the more information is gleaned about distribution of dormancy and maturity within sample. Do not delay putting seeds at storage temperature until viability has been tested. If viability is not tested immediately upon receipt, a small subsample for germination testing should be kept dry and cold (<30% RH in a refrigerator) during the lag time. Record the date the test was initiated. Seed number in initial test should be sufficient to have a statistical sampling but not so much as to deplete the sample. We suggest about 6% of the sample is used, not to exceed 200 seeds or be fewer than 15 (Guerrant and Fiedler 2004). Record the number of seeds in the assay. Record the conditions used in a germination test. When deciding the conditions to germinate seeds, there is a balance between getting an accurate count of viable seeds and gaining insights on how to induce or synchronize germination. The former informs seed banking, and the latter informs propagation and use strategies. For seed banking purposes, it is best to have standardized procedures for testing seed germination so that the conditions can be replicated in monitor tests (i.e., AOSA rules for seed testing). Viability tests should record counts for normal, abnormal, and ungerminated seeds. It is useful if ungerminated seeds are examined further to determine number of broken, empty (i.e. lack embryo), viable according to additional assessments such as vital staining, and lacking physical integrity (i.e. become mushy during imbibition). Report samples with low viability to collector or donor. Low viability samples have higher than normal proportions of damaged, empty, or mushy seeds. “Normal” proportions are determined from average values obtained from other accessions of the species reported in the literature or by genebanks.

(continued)

Table 10.4 (continued)

Standard	Explanation for difference from current FAO standard	Alternative best practice
	In current standards, flags for management decisions such as regeneration are at 85% of initial viability, which is near the detection limits of change for most seed lots. We believe this revision encourages a more statistically relevant comparison that circumvents arguments that the 85% threshold is arbitrary. Unresolved is whether large decreases in viability occur between sampling intervals.	*Viability monitoring tests* Monitor seed viability using the same conditions as initial viability assessments. Fewer seeds can be used in monitor tests; we suggest about one third of the number of seeds used in viability assessments, not to exceed 60 seeds or be fewer than 15 (Guerrant and Fiedler 2004). Record the number of seeds in the assay. Monitor samples stored under long-term conditions at intervals commensurate with their anticipated longevities: Every 40 to 50 years for long-lived species, 25–30 years for medium lived species, and 15–20 years for short-lived species. Monitor samples stored under medium-term conditions every 4–8 years depending on anticipated longevity. When the species is known to produce orthodox seed but there is no a priori knowledge of longevity, we suggest a subset of about 120 seeds be stored at 25C and 20% RH (main sample is kept at −18 °C). The subset should be monitored four times in a 3-year interval (30 seeds each). Assuming a Q10 = 2, estimate longevity achieved at −18 °C as 16 times the longevity measured in the 25C simulation and set monitoring intervals accordingly (FAO 2014).
Distributing samples	Uses of germplasm differ considerably, and efforts should be made to distribute the needed number of seeds or plant material, without overly depleting the sample.	Median number of viable propagules distributed per request should be 20–50 depending on seed size (fewer for larger seeds) and estimates of viability. Distribute seed numbers based on the users' research needs. A researcher needing an exemplar of the species should be given 5 viable propagules, and someone looking for a rare gene might get 100–200 seeds. If a researcher requires genetic material and not a viable sample, per se, consider sending them leaf tissues arising from a germination assay.

Regenerating samples	We want to stress that a germplasm bank delivers viable samples that are representative of the source population. Hence, there is an inevitable need to regenerate dying or depleted samples. Regeneration introduces high risk that genetic composition will shift. Future users must be provided with some mechanism to account for the genetic changes that have occurred in the population as a consequence of regeneration.	Regeneration plans are fundamental to germplasm banking efforts, because it is essential that live samples are provided in perpetuity. The rigor of regeneration procedures depends on the stated conservation target of the sample, with maintaining the genetic identity of samples representing populations requiring high standards in when regeneration should occur as well as selection and number of parents. Set alerts for regeneration based upon the risk of degrading the conservation target. When the goal is to maintain genetic identity of a heterogeneous population, regeneration should be scheduled when decreased viability is first detected or the sample contains fewer than 200 seeds, which ever comes first. In contrast, regeneration for a genetically homogeneous sample or a species exemplar should be based on probabilities of establishing sufficient number of plants to yield at least 2500 seeds. For wild-collected samples, recollection at the same locale can substitute for regeneration; however, it is likely that samples collected from the same wild population years apart will differ. So as not to deplete the most original sample archived in long-term storage, use it in every third or fourth regeneration, and use F1 and F2 seed for regeneration in between those cycles. If the conservation target is a representative sample of a population, a genetic analysis of the most original sample should be conducted before it is depleted. Report the number of seed-bearing plants used in a regenerated sample. Current standards recommend regenerations should incorporate a minimum of 30 (outcrossing) or 60 (selfing) plants. However, the number of parental lines can be reduced by genetic analyses that identify parental lines that will maximize capture of alleles. Use and document barriers to prevent contamination from other pollen sources.

Best practices are based on FAO genebanking standards for agriculture (FAO 2014, Chap. 4) and standards used by the Millennium Seed Bank, Kew for collecting wild species for conservation purposes (http://www.kew.org/sites/default/files/MSBP%20Seed%20Conservation%20Standards_Final%2005-02-15.pdf). Best practices suggested here are intended to address particular challenges of seeds collected from wild species as well as resolve some scientific uncertainties included in the current FAO standards usefulness of the sample, especially if the conservation target is a rare allele or an accounting of the population at a particular site and time (e.g., Franks et al. 2008). Plant genebanks can invoke certain activities to forestall, or at least understand, the extent of genetic erosion in curated samples.

10.5.1 Recollect from the Same Wild Population Over Time

Complementation of *in situ* reserves and *ex situ* collections provides a reservoir for replenishing genetic resources from wild populations (Maschinski and Haskins 2012; Guerrant et al. 2004; PCA 2015) as well as an opportunity for identifying traits of interest and how they are distributed in a natural population (e.g., Franks et al. 2008). Probabilities of mutation and fixation through drift are predicted to be lower in natural populations, compared to preserved samples, according to theoretical models (Schoen and Brown 2001). Resampling natural populations also provides the opportunity to measure the extent of genetic changes that have occurred through natural forces and those imposed by the genebank (Thormann et al. 2016; Greene et al. 2014). Programs to collect and resample after 15 years have been recently instituted and include species that are widely distributed in North America (Franks et al. 2008), some of which are priority species according to Khoury et al. (2013).

10.5.2 Regenerate Collected Seeds During Early Phases of Genebanking

Major limitations of seed accessions collected directly from wild populations are low seed number and poor seed quality. As the few available seeds age quickly, situations arise where a sample is regenerated from fewer than ten individuals, resulting in a significant bottleneck. An alternative management practice might be to immediately regenerate a newly collected sample. This would maximize the number of parents contributing to the regenerated sample as well as provide more seeds from a better growth environment and so presumably longer-lived. Studies are underway, using wild-collected germplasm of *Limnanthes*, *Humulus,* and *Artemisia*, to test this hypothesis and gain greater understanding of the interaction between storage time, seed degradation, and shifts in allelic richness for original and offspring populations created before storage and after notable degradation (unpublished; Walters, Richards, Hill, Jenderek).

Sometimes there are simply too few individuals to regenerate a population from seed. Under these circumstances, increasing sample size through clonal propagation can be effective (Pence 2013). When possible, it is important to identify the absolute number of propagules used during regeneration, as well as the number of founders from which they came: 100 individual seeds from a single maternal plant will provide a different regenerated population than one seed each from 100 different plants.

10.5.3 Use Seeds and Pollen to Back Up Wild-Collected Germplasm of Clonally Propagated Crops.

We estimate that over 30,000 accessions are maintained in living collections of the NPGS, collectively called clonal repositories. These field collections are expensive and vulnerable (Volk et al. 2015). Approximately one third of NPGS clonal accessions are CWR (Volk and Walters 2004). With current cryopreservation technologies and resources, we estimate that it will take 50–100 years to backup these accessions in preserved collections, which might not even begin until the higher priority cultivars are backed up – some 100 to 200 years from now. Clearly rapid and effective strategies to preserve these vulnerable collections are needed.

The conservation targets of CWR may be exemplars of species, snapshots of populations, or particular genes for crop improvement, but usually not the specific genotype of the collected plant. In most respects, CWR of clonal crops can be treated analogously to CWR of crops in general. Backing up these collections can be accomplished if there are seeds remaining from wild plant explorations that fit standards for quantity and quality (FAO 2014, Chap. 4 with adjustments as suggested in Table 10.4). When there are not adequate quantities of viable original seed available, trees planted in the field can serve as parents for regenerated populations. The diversity extant in these field collections can be captured through appropriate parental combinations. Feasibility of maximizing diversity with fewest crossing parents using a maximization algorithm was demonstrated (Richards et al. 2004, 2007; Volk et al. 2005), and a detailed genetic analysis shows high efficiency in capturing alleles with only minor introgression from neighboring pollen (Volk et al. 2016). Storing pollen may also be a promising method to capture and backup genetic diversity within collections (Hoekstra 1995; Volk 2011). Some CWR produce seeds that are not as amenable to conventional genebank storage conditions (i.e., they are not orthodox seeds) (Table 10.3). Methods to preserve these materials are available (Walters et al. 2013) but usually labor intensive and associated with some mortality (Wesley-Smith et al. 2014). No shifts in genetic composition were measured in recovering embryos of a high-priority CWR, *Zizania texana*, after cryoexposure (Richards et al. 2004).

10.6 Summary

Genebanks are tasked with ensuring safe preservation of genetic resources so they are available for future use. It is often difficult to predict the eventual use or the timeline for use. The challenge for plant genebanks is to provide viable germplasm that is unaffected by the genebanking experience (i.e., no genetic erosion). This mandate is harder to achieve for natural populations collected from the wild. Some wild-collected materials are likely to resist the extremely controlled conditions of the genebank and will die. Others might adapt and through drift or inadvertent selection become more domesticated. By first identifying the causes for changes in quality of germplasm in preserved collections and then offering strategies to slow down, or at least quantify, the effects of the genebanking experience, wild-collected germplasm can successfully be conserved in genebanks.

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Chapter 11
Practical Considerations for Increasing Seed Samples of Wild Species

Barbara C. Hellier

Abstract Wild species and crop wild relative samples whether for a gene bank or restoration need to be increased or replenished if original sample sizes are small, quantities have decreased with distribution and use, or viability has declined. An ideal source for fresh seed of wild species is from the original collection population. If re-collection is not possible, then ex situ increase is needed. The goal for seed increase is to maintain the genetic integrity of the original sample and produce high-quality seed. This is a challenge when growing crop species and even more of a challenge for wild species because of heterogeneity within accessions, increased seed dormancy, increased seed shattering, low seed production, indeterminant flowering and seed set, and little information on pollination biology or cultural needs. Preventing genetic drift and natural selection are two key components to maintaining genetic diversity during seed increase. A large effective population in increase plots, balanced sampling, and breaking seed dormancy are important for limiting genetic drift. Providing appropriate pollination and cultural conditions for wild species is important to impede natural selection. Seed growers can glean clues to breaking seed dormancy and the cultural needs of a species from accession passport data. Diligent attention to detail must be taken to prevent contamination between and among accessions being increased and to prevent physical damage to the seed produced through all the steps of seed production.

Keywords Seed dormancy · Genetic drift · *Elymus* · Seed production · Ex situ · Wild species

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B. C. Hellier (✉)
US Department of Agriculture – Agricultural Research Service, Plant Germplasm Introduction and Testing Research Unit, Washington State University, Pullman, WA, USA
e-mail: barbara.hellier@ars.usda.gov

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11.1 Introduction

Seed samples of wild species for use in a gene bank, restoration, or for sale are no different from cultivated species in the need to be increased or replenished if original seed sample sizes are small, quantities have decreased with distribution or use, or viability has declined. An ideal source of fresh seed for wild species samples is from the original collection population, but if re-collection is not possible due to limitations in site access or degradation and/or population decline or extinction, ex situ seed increase, frequently referred to as "regeneration" by the gene bank community, is needed. General seed production goals are to maintain the genetic integrity of the original sample and to produce high-quality seed. This goal is difficult to achieve when growing cultivated species and even more difficult for wild species. Wild species are challenging because pollination biology, mating system, germination method, cultural needs, harvest techniques, and sometimes even life form are only hinted at. Brown et al. (1997) clearly delineate the differences between domesticated and wild species attributes which contribute to the challenges. Cultivated plants have been selected for generations for uniformity – uniformity in germination, flowering time, seed set, ripening, and physical characteristics. Wild species lack that uniformity. Many of the attributes that make a species a good cultivated plant, such as low seed shattering and high seed production, are missing from wild species. Many wild species have greater seed dormancy, have longer seed production cycles, are adapted to specific habitats, are indeterminant, have greater morphological variation, and need specific pollinators compared to their domesticated cousins. All of these factors make growing wild species for seed more challenging and also more costly.

Seed production from wild and crop species requires basic knowledge about the plants selected to be grown. This includes information on the specific environment needed for growth and to trigger steps in phenology, seed dormancy and germination, growth morphology, mycorrhizal and other microbial associates, pollinators, diseases and pests, breeding system, fecundity, pollination biology, potential weediness, and potential toxins and allergens that might affect humans handling seed or plants (Sackville Hamilton and Chorlton 1997). Some of this information may be known, especially for wild species in primary crop gene pools or extensively used for restoration and revegetation, but much may need to be estimated from information on the crop or other species in the genus and the original habitat. Accurate and complete passport data that includes soil type, aspect, associated species, latitude and longitude of collection site, an estimate of total population number, and the number of plants collected, if not collected as individuals, is important for efficient and effective regeneration and seed production of wild species. While all of the above information is important, this chapter will touch on general information and practical considerations related to seed dormancy and germination, seed increase populations, stand and plot management, pollen and pollination management, harvesting seed production plots, and seed cleaning. Further resources are pointed out to readers to help in the search for species-specific information. Given the extensive experience of the author, this chapter will focus on seed production scaled to the needs of gene banks. Commercial-scale seed producers will find this information valuable as a starting point to develop strategies for new species. Gene bank regeneration differs from commercial seed production in that it is a continuous process, but with an ever-shifting cast of characters. Each year a different set of genera, species, and accessions is worked with, but the cycle is the same from year to year. Seed is germinated; seedlings are produced and transplanted to the field or greenhouse; the plants and plots are maintained; the plants flower and are pollinated; and seed is produced, harvested, and cleaned with the cycle starting over the next year with a different set of accessions. A central tenet of gene bank seed increase programs is to maintain the genetic integrity of accessions as they go through the seed increase process. Genetic change occurs through the evolutionary processes of mutation, genetic drift, and natural selection. In this chapter we attempt to point out best management practices to minimize genetic drift and natural selection during all aspects of increasing wild species.

11.2 Seed Dormancy and Germination

Seed dormancy is an adaptation to the environment to ensure germination occurs when conditions are the most favorable to growth and establishment of new plants (Finch-Savage and Leubner-Metzger 2006 and literature cited therein). Dormancy in seed is classified into two broad groups, relating to seed embryos or endogenous dormancy and exogenous dormancy, something related to the structure of the seed which prevents germination. Endogenous dormancy is further classified as either physiological, morphological, or morphophysiological (Baskin and Baskin 2001). Examples of endogenous dormancy are the need for cold or hot temperatures to stimulate germination or the need for an after ripening period where the embryo finishes growth. There are also three general classifications of exogenous dormancy: physical, chemical, and mechanical. Examples of these types of dormancy include water-impermeable seed coats, germination inhibitors in the seed endosperm, or seed having a hard wood covering (Baskin and Baskin 2001).

Many North American (NA) crop wild relative (CWR) species have varying degrees of seed dormancy (e.g., *Elymus* L. (Table 11.1), *Helianthus* L. (Chandler and Jan 1985, Table 11.1), *Zea mays* ssp. *mexicana* (Schrad.) H. H. Iltis (Avendano Lopez et al. 2011), *Solanum* L. (Spicer and Dionne 1961), *Cuphea* P. Browne (Widrlechner and Kovach 2000), *Physaria* (Nutt. ex Torr. & A. Gray) A. Gray (Von Mark et al. 2012), *Chenopodium* L. (Table 11.1), and *Penstemon* Schmidel (Allen and Meyer 1998)). This chapter does not attempt to cover germination protocols for all NA CWRs, but provides data on a set of important native species in an attempt to illustrate the complexities of effectively overcoming dormancy and germinating heterogeneous wild species (Table 11.1). Type of dormancy and amount varies widely among native genera and within genera. For example, the NA CWR species for *Triticum aestivum* L (wheat), *Elymus* and *Leymus* Hochst., are readily germinable

Table 11.1 Seed dormancy in crop wild relative species of *Triticum aestivum* (wheat), *Chenopodium quinoa* (quinoa), and *Helianthus annuus* (sunflower) and dormancy-breaking methods tried by the USDA ARS National Laboratory for Genetic Resources Preservation (NLGRP)

				Readily germinable, very low dormancy	Low dormancy	Medium dormancy	High dormancy
Crop species	Taxa (AOSA rule)[a]	NLGRP method(s) tried[b]	Total[c]	1–9% dorm.[d]	10–49% dorm.	50–89% dorm.	90–100% dorm.
Triticum aestivum	*Elymus alaskanus* (Scribn. & Merr.) Á. Löve		**3**				
		T H_2O 15–25		2			
		P GA_3 15–25		1			
	Elymus albicans (Scribn. & J. G. Sm.) Á. Löve		**0**				
	Elymus arizonicus (Scribn. & J. G. Sm.) Gould		**0**				
	Elymus bakeri (E. E. Nelson) Á. Löve	P KNO_3 15–25	**1**	1			
	Elymus canadensis L.		**27**				
	(P H_2O 15–30, pch 14 days (optional))	P H_2O 20–30		3	1		
		P H_2O 15–25		19			
		P H_2O 15–25, pch 14 days		2			
		T H_2O 15–25, pch 14 days		1			
		P H_2O 20–30, pch 14 days		1			
	Elymus curvatus Piper		**0**				
	Elymus donianus (F. B. White) Á. Löve & D. Love		**0**				
	Elymus elymoides (Raf.) Swezey		**162**				

	(P H_2O 15–30, pch 14 days (optional))	P H_2O 15		52	9	1	
		P H_2O 20		40	1		
		P H_2O 20–30		2			
		P H_2O 15–25		17	1		
		T H_2O 15		21			
		T H_2O 20		1			
		T H_2O 20–30		16	1		
	Elymus glabriflorus (Vasey ex L. H. Dewey) Scribn. & C. R. Ball	T H_2O 15	**1**	1			
	Elymus glaucus Buckley		**39**				
		P H_2O 15		2			
		T H_2O 15		5			
		P H_2O 15–25		13			
		P H_2O 20		5			
		P H_2O 20–30		10			
		T H_2O 20–30		4			
	Elymus hirsutus J. Presl		**0**				
	Elymus hystrix L.	P H_2O 20–30		1			
	Elymus interruptus Buckley		**0**				
	Elymus lanceolatus (Scribn. & J. G. Sm.) Gould		**23**				
		P H_2O 15–25		12			
		P H_2O 20–30		11			
	Elymus macgregorii R. E. Brooks & J. J. N. Campb.		**0**				

(continued)

Table 11.1 (continued)

				Readily germinable, very low dormancy	Low dormancy	Medium dormancy	High dormancy
Crop species	Taxa (AOSA rule)[a]	NLGRP method(s) tried[b]	Total[c]	1–9% dorm.[d]	10–49% dorm.	50–89% dorm.	90–100% dorm.
	Elymus macrourus (Turcz. ex Steud.) Tzvelev		**2**				
	(P H_2O 20 (new in 2016))	T H_2O 15–25		2			
	Elymus multisetus (J. G. Sm.) Burtt Davy		**29**				
		T H_2O 15		6			
		P H_2O 15–25		2			
		P H_2O 20		3			
		T H_2O 20–30		17			
		P H_2O 20–30		1			
	Elymus riparius Wiegand		**0**				
	Elymus scribneri (Vasey) M. E. Jones		**0**				
	Elymus sierrae Gould		**0**				
	Elymus stebbinsii Gould		**0**				
	Elymus trachycaulus (Link) Gould ex Shinners		**166**				
	(P H_2O 20–30, pch 5 days (optional))	P H_2O 15			1		
		P H_2O 15–25		39			
		B H_2O 15–30		4			
		P H_2O 20–30		110			
		T H_2O 20–30		6			
		P H_2O 20–30, pch 5 days		6			

	Elymus violaceus (Hornem.) Feilberg		**0**				
	Elymus virginicus L.		**12**				
		P H_2O 15–25		2			
		T H_2O 20		1			
		P H_2O 20–30		6	2		
		T H_2O 20–30			1		
	Elymus wawawaiensis J. R. Carlson & Barkworth		**26**				
	(P H_2O 20 (new in 2016))	P H_2O 15–25		9			
		T H_2O 15–25		15			
		P H_2O 20–30		2			
	Elymus wiegandii Fernald		**0**				
	Leymus ajanensis (V. N. Vassil.) Tzvelev		**0**				
	Leymus ambiguus (Vasey & Scribn.) D. R. Dewey		**0**				
	Leymus californicus (Bol. ex Thurb.) Barkworth		**0**				
	Leymus cinereus (Scribn. & Merr.) Á. Löve		**184**				
	(P H_2O 15–25)	P H_2O 15–25		135	31		
		T H_2O 15–25		6	9		
		P H_2O 20–30		1			
		T H_2O 20–30		1	1		
	Leymus condensatus (J. Presl) Á. Löve		**3**				
		P H_2O 15–25		2			
		P H_2O 20–30		1			

(continued)

Table 11.1 (continued)

				Readily germinable, very low dormancy	Low dormancy	Medium dormancy	High dormancy
Crop species	Taxa (AOSA rule)[a]	NLGRP method(s) tried[b]	Total[c]	1–9% dorm.[d]	10–49% dorm.	50–89% dorm.	90–100% dorm.
	Leymus flavescens (Scribn. & J. G. Sm.) Pilg.	T H_2O 15–25	**1**		1		
	Leymus innovatus (Beal) Pilg.		**6**				
		P H_2O 15–25		1			
		T H_2O 15–25		1			
		P H_2O 20–30		4			
	Leymus mollis (Trin.) Pilg.		**6**				
		P H_2O 15–25		3			
		T H_2O 15–25		1			
		P H_2O 20–30		1			
	Leymus pacificus (Gould) D. R. Dewey		**0**				
	Leymus salina (M. E. Jones) Á. Löve		**4**				
		P H_2O 15–25		1	1	1	
		T H_2O 15–25		1			
	Leymus simplex (Scribn. & T. A. Williams) D. R. Dewey		**0**				
	Leymus triticoides (Buckley) Pilg.	P H_2O 15–25	**8**	3	5		
Chenopodium quinoa	*Chenopodium berlandieri* Moq.		**36**				
		P H_2O 15–25		9	4	3	
		P H_2O 20–30		2	2	9	1
		P KNO_3 20–30		2		2	
	Chenopodium neomexicanum Standl.		**7**				

		P H_2O 15–25		1	3	1	
		P H_2O 20–30				1	1
	Chenopodium standleyanum Aellen		**2**				
		P H_2O 20–30					1
		P KNO_3 20–30					1
	Chenopodium watsonii A. Nelson		**4**				
		P H_2O 20–30					3
		P H_2O 15–25				1	
Helianthus annuus	*Helianthus anomalus* S. F. Blake		**5**				
	(B or T H_2O 20–30)	T H_2O 20					2
		T H_2O 20–30					3
	Helianthus argophyllus Torr. & A. Gray		**51**				
	(B or T H_2O 20–30)	T H_2O 20		10	5		
		T H_2O 20–30		19	15	2	
	Helianthus bolanderi A. Gray		**27**				
	(B or T H_2O 20–30)	T H_2O 20				2	1
		P GA_3 20–30			1		
		T H_2O 20–30			1	6	15
		T H_2O 20–30, 14-day pch					1
	Helianthus debilis Nutt.		**31**				
	(B or T H_2O 20–30)	T H_2O 20		2	3	1	
		T H_2O 20–30			2	12	11
	Helianthus deserticola Heiser		**16**				
	(B or T H_2O 20–30)	T H_2O 20				1	2

(continued)

Table 11.1 (continued)

				Readily germinable, very low dormancy	Low dormancy	Medium dormancy	High dormancy
Crop species	Taxa (AOSA rule)[a]	NLGRP method(s) tried[b]	Total[c]	1–9% dorm.[d]	10–49% dorm.	50–89% dorm.	90–100% dorm.
		T H_2O 20–30					13
	Helianthus neglectus Heiser		**24**				
	(B or T H_2O 20–30)	T H_2O 20				5	
		T H_2O 20–30				10	9
	Helianthus niveus (Benth.) Brandegee		**23**				
	(B or T H_2O 20–30)	T H_2O 20		2	1	1	1
		T H_2O 20–30		5	5	2	6
	Helianthus paradoxus Heiser		**12**				
	(B or T H_2O 20–30)	T H_2O 20		1	5	3	
		T H_2O 20–30				3	
	Helianthus petiolaris Nutt.		**27**				
	(B or T H_2O 20–30)	T H_2O 20	**3**			2	1
		T H_2O 20–30	**19**			7	12
		T GA_3 20–30	**1**			1	
		T H_2O 20–30 with H_2O_2 Trt	**4**			2	2
	Helianthus praecox Engelm. & A. Gray						
	(B or T H_2O 20–30)	T H_2O 20–30	**4**			4	
	Helianthus angustifolius L.		**21**				
	(B or T H_2O 20–30)	T H_2O 20	**10**		1	1	8
		T H_2O 20–30	**11**		1	7	3
	Helianthus arizonensis R. C. Jacks.						

	(B or T H_2O 20–30)	T H_2O 20–30	**2**			1	1
	Helianthus atrorubens L.		**14**				
	(B or T H_2O 20–30)	T H_2O 20	**1**			1	
		T H_2O 20–30	**13**		1	3	9
	Helianthus californicus DC.						
	(B or T H_2O 20–30)	T H_2O 20–30	**14**		3	5	6
	Helianthus ciliaris DC.						
	(B or T H_2O 20–30)	T H_2O 20–30	**5**		1		4
	Helianthus cusickii A. Gray		**19**				
	(B or T H_2O 20–30)	T H_2O 20–30	**18**			3	15
		P GA_3 20–30	**1**		1		
	Helianthus decapetalus L.		**15**				
	(B or T H_2O 20–30)	T H_2O 20	**2**		1	1	
		T H_2O 20–30	**13**		1	1	11
	Helianthus divaricatus L.		**12**				
	(B or T H_2O 20–30)	T H_2O 20	**1**				
		T H_2O 20–30	**10**		1		9
		T H_2O 20–30, 14-day pch	**1**			1	
	Helianthus eggertii Small						
	(B or T H_2O 20–30)	T H_2O 20–30	**11**				11
	Helianthus floridanus A. Gray ex Chapm.						
	(B or T H_2O 20–30)	T H_2O 20	**3**		1		2
		T H_2O 20–30	**3**			2	1
		T H_2O 20–30, 28-day pch	**1**		1		

(continued)

Table 11.1 (continued)

				Readily germinable, very low dormancy	Low dormancy	Medium dormancy	High dormancy
Crop species	Taxa (AOSA rule)[a]	NLGRP method(s) tried[b]	Total[c]	1–9% dorm.[d]	10–49% dorm.	50–89% dorm.	90–100% dorm.
	Helianthus giganteus L.		**20**				
	(B or T H_2O 20–30)	T H_2O 20	**4**		1		3
		T H_2O 20–30	**5**			4	1
		T H_2O 20–30, 14-day pch	**7**	1	2	4	
		T H_2O 20–30, 28-day pch	**4**	1	2	1	
	Helianthus glaucophyllus D. M. Sm.		**8**				
	(B or T H_2O 20–30)	T H_2O 20	**1**				1
		T H_2O 20–30	**7**			3	4
	Helianthus gracilentus A. Gray		**12**				
	(B or T H_2O 20–30)	P H_2O 20	**1**				1
		T H_2O 20–30	**10**			1	9
		T H_2O 20–30, 14-day pch	**1**				1
	Helianthus grosseserratus M. Martens		**36**				
	(B or T H_2O 20–30)	T H_2O 20	**12**	4	8		
		T H_2O 20–30	**8**	1	3	3	1
		T H_2O 30–30, 14-day pch	**7**		2	5	
		T H_2O 20–30, 28-day pch	**9**	1	7	1	
	Helianthus hirsutus Raf.		**7**				
	(B or T H_2O 20–30)	T H_2O 20	**2**		1		1

		T H_2O 20–30	**1**			1	
		T H_2O 30–30, 14-day pch	**2**			1	1
		T H_2O 20–30, 28-day pch	**2**			1	1
	Helianthus laciniatus A. Gray						
	(B or T H_2O 20–30)	T H_2O 20–30	**6**		2	1	3
	Helianthus laevigatus Torr. & A. Gray		**4**				
	(B or T H_2O 20–30)	T H_2O 20	**1**				1
		T H_2O 20–30	**2**				2
		T H_2O 20–30, 28-day pch	**1**		1		
	Helianthus longifolius Pursh		**2**				
	(B or T H_2O 20–30)	T H_2O 20	**1**				1
		T H_2O 20–30	**1**			1	
	Helianthus maximiliani Schrad.		**20**				
	(B or T H_2O 20–30)	T H_2O 20	**1**			1	
		T H_2O 20–30	**16**	6	1	6	3
		T H_2O 30–30, 14-day pch	**2**			2	
		T H_2O 20–30, 28-day pch	**1**		1		
	Helianthus microcephalus Torr. & A. Gray		**9**				
	(B or T H_2O 20–30)	T H_2O 20	**1**				1
		T H_2O 20–30	**4**				4

(continued)

Table 11.1 (continued)

				Readily germinable, very low dormancy	Low dormancy	Medium dormancy	High dormancy
Crop species	Taxa (AOSA rule)[a]	NLGRP method(s) tried[b]	Total[c]	1–9% dorm.[d]	10–49% dorm.	50–89% dorm.	90–100% dorm.
		T H_2O 20–30, 28-day pch	**4**		1	3	
	Helianthus mollis Lam.		**18**				
	(B or T H_2O 20–30)	T H_2O 20	**2**			1	1
		T H_2O 20–30	**10**		1		9
		T H_2O 30–30, 14-day pch	**4**		2	2	
		T H_2O 20–30, 28-day pch	**2**			2	
	Helianthus nuttallii Torr. & A. Gray		**30**				
	(B or T H_2O 20–30)	T H_2O 20	**9**	1	4	2	2
		T H_2O 20–30	**17**		2	12	3
		P H_2O 20–30	**1**				1
		T H_2O 20–30, 14-day pch	**1**			1	
		T H_2O 20–30, 28-day pch	**2**		2		
	Helianthus occidentalis Riddell		**16**				
	(B or T H_2O 20–30)	T H_2O 20	**5**	1	4		
		T H_2O 20–30	**11**		2	4	5
	Helianthus pauciflorus Nutt.		**31**				
	(B or T H_2O 20–30)	T H_2O 20	**3**			1	2
		T H_2O 20–30	**19**			2	17

		T H_2O 20–30, 14-day pch	**9**			9	
	Helianthus pumilus Nutt.		**49**				
	(B or T H_2O 20–30)	T H_2O 20	**3**			1	2
		T H_2O 20–30	**44**			3	41
		T GA_3 20–30	**1**			1	
		P H_2O 20–30	**1**				1
	Helianthus resinosus Small		**15**				
	(B or T H_2O 20–30)	T H_2O 20	**3**			1	2
		T H_2O 20–30	**12**			5	7
	Helianthus salicifolius A. Dietr.						
	(B or T H_2O 20–30)	T H_2O 20–30	**6**				6
	Helianthus schweinitzii Torr. & A. Gray						
	(B or T H_2O 20–30)	T H_2O 20–30	**1**				1
	Helianthus silphioides Nutt.		**13**				
	(B or T H_2O 20–30)	T H_2O 20	**3**			1	2
		T H_2O 20–30	**10**			8	2
	Helianthus simulans E. Watson		**5**				
	(B or T H_2O 20–30)	P KNO_3 20	**1**			1	
		T H_2O 20	**1**			1	
		T H_2O 20–30	**3**			2	1
	Helianthus smithii Heiser						
	(B or T H_2O 20–30)	T H_2O 20–30	**6**			1	5
	Helianthus strumosus L.						
	(B or T H_2O 20–30)	T H_2O 20	**1**		1		
		T H_2O 20–30	**8**			2	6

(continued)

Table 11.1 (continued)

				Readily germinable, very low dormancy	Low dormancy	Medium dormancy	High dormancy
Crop species	Taxa (AOSA rule)[a]	NLGRP method(s) tried[b]	Total[c]	1–9% dorm.[d]	10–49% dorm.	50–89% dorm.	90–100% dorm.
		T H_2O 20–30, 14-day pch	**2**	1		1	
	Helianthus tuberosus L.						
	(B or T H_2O 20–30)	T H_2O 20	**2**			1	1
		T H_2O 20–30	**33**		1	14	18
		T H_2O 20–30, 14-day pch	**2**	1		1	

Source: AL Miller, USDA ARS National Laboratory for Genetic Resources Preservation, Plant Germplasm Resources Preservation Program, Fort Collins, CO

[a] Information in parentheses is the Association of Official Seed Analyst test rule. If no information is present, there are no rules for the species

[b] Method codes: Substrate: *B* between blotters, *T* germination towels, *P* petri dish (or on top of blotters in a germination box). Moisture (substrate moistened with): H_2O tap water, KNO_3 0.2% solution of potassium nitrate, GA_3 500 ppm gibberellic acid solution, H_2O_2 24-h soak in hydrogen peroxide. Temperature: 20 = constant 20 °C; 20–30 = alternating 20 °C night to 30 °C day (8 h); 15–25 = alternating 15 °C night to 25 °C day (8 h). Stratification: pch = 5 °C on moist medium for specified period of days

[c] The total number of samples tested by the NLGRP

[d] The number of samples tested. Dormancy is based on the proportion of total viable seed that were dormant as measured by a TZ test on the remaining ungerminated seed at the end of the germination period

except *Elymus elymoides* (Raf.) Swezey and *Leymus salina* (M. E. Jones) Á. Löve, which have low levels of dormancy (Table 11.1). Most of the *Chenopodium* and *Helianthus* species have medium to high levels of dormancy in stored seed samples (Table 11.1). This differs greatly from the cultivated species for both of these genera which germinate readily. Dormancy can also vary within a species depending on collection location. Dormancy in populations of *Penstemon eatonii* A. Gray varied from no dormancy in seed collected at low elevation sites to a 24-week chilling requirement for seed from high elevation sites (Meyer 1992).

Identifying environmental characteristics that impose risks or unfavorable conditions to newly germinated seed and seedlings is important for understanding how to break dormancy (Allen and Meyer 1998). For example, the habitat risks facing *Penstemon eatonii*, native to the Intermountain Northwest, and Mexican teosinte populations from low elevation sites are very different. The primary abiotic risks for *P. eatonii* are drought and frost (low temperature) (Allen and Meyer 1998), but for teosinte it is humidity and high temperature (Avendano Lopez et al. 2011). Accurate and complete passport data, especially latitude and longitude of the collecting site, are crucial for pinpointing the habitat risks when the type of dormancy for a particular species or accession has not been determined experimentally. It is important to determine how to break dormancy since regenerating a seed lot with a mix of dormant and nondormant seeds can lead to unintended selection, changing the genetics of the accession (Sackville Hamilton and Chorlton 1997).

There are several databases with wild species germination protocols that include NA native species (e.g., Native Plant Network (http://npn.rngr.net/propagation/protocols), USDA-NRCS PLANTS database (http://plants.usda.gov/), and Kew Royal Botanic Gardens, Millennium Seed Bank Partnership, Seed Information Database (http://data.kew.org/sid/)). Also, the Association of Official Seed Analysts (AOSA 2016) and International Seed Testing Association (ISTA 2017) rules for testing seed may have protocols for some wild species, but germination protocols for many are not available. If time and an adequate supply of seed (450+) are available, Baskin and Baskin (2003) have developed a "move-along experiment" to help determine the conditions for breaking dormancy for species with water-permeable seed. This experiment takes 1 year. Seed samples are moved through temperatures simulating winter, spring, summer, and fall with controls placed at the winter and summer temperatures. This determines the optimum temperature or combination of temperatures needed for germination. They recommend using fresh seed, but Kew has successfully used dried, cleaned, and stored seed for this experiment (Hay and Probert 2013). If time and seed supplies are limited, monthly temperatures and rainfall patterns for the collection location can be used to predict optimal germination temperatures and dormancy-breaking treatments (Hay and Probert 2013; Davies et al. 2015). Climate data can be obtained from the National Oceanic and Atmospheric Administration, National Centers for Environmental Information website (https://www.ncei.noaa.gov/), and WorldClim (http://www.worldclim.org/) for sites outside the USA. Prior to initiating lengthy tests like the "move-along" experiments, seed should be tested for fill and viability using tetrazolium tests. This establishes a baseline for evaluating the efficacy of the experiments or other dormancy-breaking treatments. It also avoids wasting time and effort conducting experiments on low-quality seed.

11.3 Minimizing Genetic Change in the Seed Increase Population

When more seed is needed of native species, the ideal, in terms of minimizing artificial genetic change, is to re-collect from the original population, which may have hundreds if not thousands of plants. Guidelines for sampling wild populations to minimize negative impacts are covered in Chap. 8. Re-collection from wild populations can be less expensive and has the added benefit of capturing genetic change due to natural evolutionary processes over time. However, seed quality of wild collected germplasm may be compromised by insects, drought, or other factors. Frequently re-collection is not possible, and ex situ seed increase is needed. There are many points in the seed increase process where the genetic integrity of an accession can be compromised. Physical contamination can occur when seed is packaged for grow-out, seed is started, seedlings are transplanted, and seed is harvested, cleaned, and stored. These can be controlled through appropriate operational procedures to ensure no physical contamination takes place. Contamination can also occur during pollination; later in this chapter, we will discuss how pollination contamination can be controlled. The most difficult types of genetic change to control are those changes that can occur through random genetic drift (sampling variation) and natural selection (Sackville Hamilton and Chorlton 1997; Lawrence 2002; van Hintum et al. 2002; Richards et al. 2010). A good discussion of the mechanisms of both can be found in Sackville Hamilton and Chorlton (1997). Natural selection can be minimized by overcoming seed dormancy, using high-quality seed, conducting multiple seed harvests, and insuring production methods optimize growth of all individual plants. Key components of the seed increase process to minimize genetic drift are the number of plants in the increase population and seed sampling or harvesting strategies. Minimizing random drift can primarily be achieved by using an adequate number of plants in the seed increase population. The number of plants used for increase is influenced by the mating system of the species and the heterogeneity of the accession. Generally, smaller increase populations can be used for autogamous species and/or homozygous accessions; larger plant numbers are needed for outcrossing species and/or heterogeneous accessions. Genebank Standards (FAO/IPGRI 1994) recommend a minimum of 100 plants be used for seed increase. Lower and higher numbers have also been recommended. For example, Breese (1989) recommended an effective population (N_e) size, the number of individuals actually contributing to the next generation, be 25. Two hundred ten individuals were recommended to maintain alleles with a frequency of 0.05 or greater with 90% probability by Crossa et al. (1993). Experimental data measuring the effects of regeneration on allele frequency and heterozygosity is only available for a few crops related to important NA CWRs and fewer NA wild species. For cultivated carrot (*Daucus carota* L. subsp. *sativus* (Hoffm.) Schubl. & G. Martens var. *sativus* Hoffm.), Le Clerc et al. (2003) tested populations of 2–70 plants and found allele frequencies changed the least when 70 plants were used for seed increase if balanced samples (i.e., the same number of seed is harvested from each plant in the population) were collected. Even a large population of 120 *Phaseolus vulgaris* L. (common bean) plants of a landrace was not enough to maintain the allelic diversity of the material when compared to on-farm in situ populations with thousands of plants (Negri and Tiranti 2010). Johnson et al. (2002, 2004) looked at the effect of harvest method on allele frequencies in three wind-pollinated perennial grasses (*Festuca pratensis* Huds. (English bluegrass), *Lolium perenne* L. (English ryegrass), and *Pseudoroegneria spicata* (Pursh) Á. Löve (bluebunch wheatgrass)). Balanced sampling reduced allele frequency shift to 4% as compared to the original seed. In all these studies, the calculated N_e was less than the census population (N_c) requiring additional plants to be planted to achieve the desired N_e. For carrot, an additional 30 plants were needed to achieve a N_e of 50, and for grasses, 50 additional plants were needed. Because wild species are more heterogeneous than their domesticated congeners, the number of plants in the increase population should be greater than guidelines developed for domesticated species.

The best harvest or sampling strategy to conserve rare alleles and maintain allele frequency of the original material is to harvest and maintain seed from each plant in the increase population separately (Breese 1989; Lawrence 2002) or harvest a balanced sample by collecting the same number of seed from each plant in the increase population (Brown et al. 1997; Le Clerc et al. 2003). This ensures that one or a few individuals do not over contribute to the next generation and that maternal contribution is equal. This is more important for heterogeneous accessions, such as wild species, than for more homogenous ones. The same authors also suggest keeping two samples for each accession: a composite inventory of individually harvested plants maintained as regeneration seed stock and a bulk harvest seed lot maintained for distribution and use. Ideally, the bulked inventory would be a balanced sample. For grasses, Johnson et al. (2004) found that sampling two to three inflorescences per plant provided similar benefits for increasing the ratio of N_e/N_c as harvesting the same number of seed per plant. The above strategies are ideal to minimize genetic change during the seed increase process. As with many other aspects of wild seed production, resources are frequently insufficient to carry out these harvest strategies.

The viability of the sample used for seed increase also contributes to maintaining accession genetic diversity as demonstrated through modeling by Richards et al. (2010); samples with lower viability rates lost diversity faster through successive regenerations than those with higher viability rates, and the first increase generation is the most important. If the initial population is sufficient to minimize diversity loss, subsequent regenerations will be more successful at maintaining the remaining diversity (Richards et al. 2010). Dulloo et al. (2008) suggest that samples be regenerated before they decline to 61–64% viability for wild species and that the most original seed lot should be used. Effective seed storage can help minimize regeneration frequencies by ensuring seed is stored under conditions that maintain longevity. Within the USDA ARS National Plant Germplasm System (NPGS), many gene banks are storing seed at −18 ° C. Regeneration stocks of short-lived species may benefit from being stored in liquid nitrogen vapor.

Maintaining diversity is most challenging in accessions that are heterogeneous and/or of outcrossing species. This includes most wild species. It is important to remember that the number of individuals actually contributing to the allelic diversity of the next generation is generally less than the census population and the largest population size feasible should be used for seed increase. A compromise is usually necessary between the ideal population size and what is economically affordable in terms of labor cost to plant, maintain, harvest, and clean seed and available physical resources including land. Because of this, it is imperative that a record be maintained indicating the number of plants harvested so users of the increased seed lot understand that genetic integrity may have been compromised.

11.4 Stand and Plot Management

With the increased interest in using native species for restoration and revegetation (Great Basin Restoration Initiative 1999; Seeds of Success 2001; Gerling et al. 1996), the cultural requirements of more and more natives are known. There are a number of native plant guides for regions and states across the country (Horton et al. 1990; Stevens et al. 1996; Rose et al. 1998; Pfaff et al. 2002; Burton and Burton 2003; Tucson PMC 2004; Godin 2007; Harper et al. 2007; Houseal 2007; Rancho Santa Ana Botanic Garden 2016; Bartow 2015), and the number of propagation protocols in the Native Plant Network database (http://www.nativeplantnetwork.org/) is growing. Most of the species in the guides and databases are not CWRs, but there are a few (e.g., *Zizania aquatica* L., species of *Elymus*, *Leymus*, *Helianthus*, *Lomatium* Raf.). The general cultural information in these guides also applies to CWRs. The most common recommendation in all guides is to start with a weed-free field. Weed control is the largest expense for native seed producers (Anderson 1999). Using herbicides is not recommended because the phytotoxicity of most herbicides is not known for wild species and even if they are registered for a related crop, herbicides may kill the wild relative. Herbicides can impact seed production and/or flowering even if they do not kill the plants (Burton and Burton 2003).

Minimizing natural selection in seed increase populations is as important as reducing genetic drift for maintaining genetic integrity (Sackville Hamilton and Chorlton 1997). It is necessary to select cultural conditions to optimize plant survival, flowering, and seed production of all plants in the population to achieve this. Complete passport data is important to help determine the best grow-out conditions for wild species. It can help determine the soil, moisture, and photoperiod needed for a particular species or accession. Latitude and longitude can be used to find collecting site information on climate or soil using environmental data sets such as WorldClim. Another useful tool are seed zone maps; maps which indicate an area within which plant materials can be transferred with less risk of being poorly adapted to their new location (http://www.fs.fed.us/wwetac/threat_map/SeedZones_Intro.html). Species-specific seed zone maps have been developed for a few herbaceous NA native species important for restoration (Johnson et al. 2010, 2012, 2013; St Clair et al. 2013) along with a general provisional map for native plants (Bower et al. 2014).

Using transplants or plugs to establish seed increase plots may help equalize survival for all genotypes in an accession, and providing adequate and equal spacing within the plot reduces competition for resources (Breese 1989). The common assumption is that wild populations are resource limited (water, light, or nutrients). Optimizing resources may increase seed production in some wild species (Wilson and Price 1980; Shock et al. 2012), but it is not always the case (Mueller et al. 2000). Many of the production guides recommend limiting fertilizer application to help control weeds.

11.5 Pollen and Pollination Management

Outcrossing species need isolation to prevent contamination from alien sources of pollen in order to maintain genetic integrity during seed increase. This can be achieved through caging that excludes insect pollinators (Fig. 11.1) or wind-borne pollen or spatial or temporal isolation. Pollination biology is known for a few NA CWRs with most being either anemophilous, e.g., grasses and varieties of *Zizania* (Lu et al. 2005), or entomophilous, e.g., species of *Physalis* (Sullivan 1984), *Ipomoea* L. (Real 1981), and *Echinacea* Moench (Stephens 2008; Ison et al. 2014). Limited literature is available providing specifics on pollination methods that can be used for NA CWRs. Brenner and Widriechner (1998) provide details on using small plastic chambers for *Amaranthus* L. species regeneration in greenhouses. For cultivated species, various pollinators have been used in pollination cages and greenhouses. The most common are bee, bumble bee, and fly species. Honey bees, *Apis mellifera* L., and blowflies (species in C*alliphora* and *Lucilia*) (Fig. 11.1) are used in onion seed cages (Currah and Ockendon 1984).

Alfalfa leaf cutter bees (*Megachile rotundata* Fabricius) were found to be the most cost-effective pollinators in caged seed production of species of *Trifolium* L., *Medicago* L., and *Lotus* L. (Greene and Bell 2007). *Calliphora vicina* Robineau-Desvoidy (European blue blowfly) are effective in carrot (Howlett 2012) and leek seed production cages (Clement et al. 2007), and hoverfly species *Episyrphus balteatus* de Geer and *Eristalis tenax* L. can be used for oilseed rape (*Brassica napus* L.) (Jauker and Wolters 2008) and turnip (*Brassica rapa* L.) cage pollination (Schittenhelm et al. 1997) and sweet peppers in the greenhouse (Jarlan et al. 1997). It is now common practice to use *Bombus* (bumble bees) species to pollinate greenhouse grown tomatoes (Velthuis and van Doorn 2006). A combination of red mason bees (*Osmia rufa* L.) and hover flies have been used for greenhouse

Fig. 11.1 (**a**) *Allium acuminatum* Hook. in pollinator exclusion cages made from bent rebar and window screen; blowflies were used as pollinators. (**b**) Blowfly pollinator on a *Lomatium dissectum* (Nutt.) Mathias & Constance umbel. (**c**) Insect exclusion cage over a *Lactuca sativa* L. seed increase plot (the door to the cage is open for harvest)

pollination with a mixture of several genera (carrot, onion, celery, dill, kale, radish) (Gladis 1996). The USDA ARS North Central Plant Introduction Station, Ames, IA, reported using *Osmia cornifrons* Radoszkowski, for oilseed *Brassica*, *Bombus bimaculatus* Cresson for snapdragons, and *Musca domestica* L. for Apiaceae in conjunction with honey bee in field regeneration cages (Widrlechner et al. 1996) and honey bees and *Osmia* sp. in *Melilotus* (L.) Mill. regeneration cages (Brenner 2005).

Since the most effective pollinator for wild species usually is not known, using spatial isolation and allowing native pollinators to pollinate may be the best option for wild species. Clement et al. (2006) found 52 species of bees, primarily species of native solitary bees (*Osmia*) and bumble bees (*Bombus*) visiting seed increase plots of *Astragalus* L. and *Onobrychis* Mill. at the USDA ARS Western Regional Plant Introduction Station Central Ferry Farm in Washington. It may benefit seed producers to enhance habitat for native pollinators around their farms (Klein et al. 2007). This can be done by leaving gaps in surface vegetation for nesting (Shuler et al. 2005), retaining neighbor forest sites if available (Cane 1997), leaving dead wood for cavity nesting species (Westrich 1996), and planting or providing diverse floral resources (Ghazoul 2006). With the global pollinator decline (Potts et al.

2010), this would not only provide pollinators for on-farm seed production but aid in native pollinator conservation. Spatial isolation distances needed for native pollinators may be unknown, but spatial isolation distances used in commercial production of crop species can be used as a guide. For example, in alfalfa (*Medicago sativa* subsp. *sativa* L.), isolation distances of 274 m, 1.6 km, and 4.8 km are recommended when leaf cutter bees, alkali bees (*Nomia melanderi* Cockerell), or honey bees, respectively, are used as pollinators (Van Deynze et al. 2008).

Wind-pollinated species require caging with pollen-proof material or spatial isolation. Isolation distance will change depending on the species. Johnson et al. (1996) found that an isolation distance of 22–27 m with the presence of an abundance of non-contaminating pollen was an effective barrier to minimizing contaminating pollen for *Bromus inermis* Leyss. In maize, no pollination occurred 300 m from the pollen source (Luna et al. 2001). Song et al. (2003) observed a maximum gene flow distance of 43.2 m from cultivated rice to the wild species *Oryza rufipogon* Griff.

11.6 Harvesting Seed Production Plots and Seed Cleaning

Gene bank seed production plots are typically small populations. Because of the small plant numbers, harvest and cleaning tend toward hand methods or equipment modified for small seed lots. Also, for genetic integrity conservation, the best sampling strategy of harvesting equal numbers of seed from each plant precludes using mechanical harvesters except when collecting bulk samples. The challenge for all species is determining what the most efficient method is for each (e.g., rubbing seed from stalks or heads, clipping whole seed stalks, stripping pods from stalks, harvesting whole plants) and the timing of harvest, especially relevant for wild species because of indeterminate seed ripening and shattering. Often, multiple harvests are needed, or plants are planted into plastic mulch so seed can be collected from the ground, or a harvest date is chosen which is a compromise between some seed shattering and some immature seed being harvested.

Many of the production guides previously mentioned give specific harvest and cleaning methods for the native species they include. These are a good starting point for related or similar species. They provide the best harvest method, information on shattering, timing of seed maturity, and cleaning methods. Because most guides are geared toward large production plots, mechanical harvesting methods are featured with information on combining, swathing, strippers, and vacuum harvesting. The Revegetation Equipment Catalog (http://reveg-catalog.tamu.edu/) has information on equipment and suppliers for harvesting and seed processing equipment, but again, it mostly features equipment for large plot harvesting. For small plot harvest, often times gene bank personnel modify or repurpose equipment to fit the niche need. Figure 11.2 shows a “D-Vac” designed for entomological sampling which has been modified for small plot harvest of Asteraceae and Onagraceae seed with a pappus or fluff. Whatever harvest method is used, it needs to prevent seed from

Fig. 11.2 Modified "D-Vac" for use in harvesting small plots of Asteraceae, Onagraceae, or other species seed with a pappus or fluff. Seed is vacuumed into a fine screen "sock" at the end of the flexible harvest hose. The sock and hose are cleaned between plots, so there is no cross-plot contamination

spreading between plots during harvest and provide zero carry-over from plot to plot in the harvester.

Production guides also provide details on seed conditioning and cleaning equipment. At the USDA ARS Western Regional Plant Introduction Station, Pullman, WA, air column and gravity separators along with hand sieves with various shape and size screens are staple tools for cleaning seed of wild species which are often small and light. Additional cleaning and conditioning equipment used includes brush machines, velvet rollers, hammermills, air screen cleaners, debearder, scarifier, and rubbing boards. The Tucson PMC Native Plant Guide (Tucson Plant Material Center 2004) has detailed descriptions of these machines and how they work. The debearder, scarifier, and rubbing board should be used with caution to prevent mechanical damage (abrasions and breakage). Such damage can be lethal especially in seeds that have embryos close to the seed coat surface or radicles at acute tips of seeds. Abrasions also provide entry points for pathogens and reduce longevity of seeds in storage. Magnification should be used at various steps throughout the cleaning process to assess and minimize the impact of cleaning efforts. As with all other steps in the seed production process, detailed attention needs to be given to cleaning all equipment between accessions to prevent contamination and assure genetic integrity.

11.7 Conclusion

The goals for ex situ seed production of wild species are to produce high-quality seed and to maintain the genetic integrity of the original material. In order to achieve these goals, information on each species needs to be obtained either through experimental work or gleaned from the specific collection location or general species habitat. Maintaining the genetic diversity of the material grown requires knowledge of germination and dormancy-breaking protocols, cultural requirements, pollination biology, and phenology of the species. Diligent attention to detail must be taken to prevent contamination between and among accessions being grown and to prevent physical damage to the seed produced through all the steps of seed production – germination, seedling production, stand management, pollination, harvest, and cleaning. The number of plants in a regeneration population is one of the most important factors in maintaining the alleles present in the original material. It is critical to remember that the effective population (the number of individuals actually contributing gametes to the subsequent generation) is usually smaller than the census population and plant numbers need to be increased to accommodate this. Another key component to maintaining genetic integrity is the sampling strategy used during harvest with the most effective being to harvest equal numbers of seed from each individual plant of the seed increase population. Although this chapter outlines best practices aimed at minimizing genetic change during the seed increase process, rarely are fiscal and infrastructure resources sufficient for full implementation. In this case, seed producers need to evaluate trade-offs and identify the best possible methods that allow them to reach their objectives by drawing upon innovative solutions.

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Chapter 12
Public Education and Outreach Opportunities for Crop Wild Relatives in North America

Tara Moreau and Ari Novy

Abstract Successful programs of crop wild relative (CWR) exploration, conservation, and utilization are ultimately dependent on sustained public prioritization and support, which in turn requires public awareness and engagement. Here we discuss the importance of advancing North America public education and outreach activities related to CWR, including improving capacity for public engagement, stakeholder building, partnering for education and outreach, and programmatic development. We focus specifically on the potential of botanic gardens as excellent partners for public engagement on CWR due to their presence in major population centers, knowledge of informal educational practices, and familiarity with plant biodiversity and agricultural crop species. We also discuss CWR outreach efforts outside of North America as well as related environmental education efforts within the region. This chapter provides an informal education and outreach primer for researchers who want to incorporate public engagement into their CWR research programs, as well as for informal education professionals seeking to capitalize on the growing public interest in food systems to explore food-related biodiversity topics.

Keywords Agricultural education · Broader impacts · Capacity building · Informal education · Germplasm education · Botanical Gardens

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T. Moreau (✉)
University of British Columbia Botanical Garden, Vancouver, BC, Canada
e-mail: tara.moreau@ubc.ca

A. Novy
Leichtag Foundation, Encinitas, CA, USA

National Museum of Natural History, Smithsonian Institution, Washington, DC, USA

University of California-San Diego, San Diego, CA, USA

San Diego Botanic Garden, Encinitas, CA, USA
e-mail: anovy@sdbgarden.org

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12.1 Introduction

In the face of a changing climate, prioritizing the exploration, conservation, and utilization of crop wild relatives (CWR), and food plant genetic resources in general, is vital for future food security. As the ancestors and relatives of our food plants, CWR are important sources of genetic diversity because they have adapted to survive in many different soil, pest, disease, and growing conditions. They have tremendous breeding value for food plant productivity, nutrition, and sustainability. However, the planet's sixth mass extinction of species is underway and one in five plant species threatened (Ceballos et al. 2015; Royal Botanic Gardens Kew 2016). Currently, CWR are underrepresented in genebanks and threated in their native habitats (Castañeda-Álvarez et al. 2016; Hunter and Heywood 2011).

Agricultural scientists dedicated to the exploration, conservation, and use of CWR can increase the impact of their work through collaborations with public education and outreach communities. Public engagement and education are key deliverables in international conservation frameworks (e.g., Global Strategy for Plant Conservation and UN Sustainable Development Goals) (Convention on Biological Diversity 2012; United Nations 2017). At local levels, public engagement can raise the profile of CWR as part of broader agendas on biodiversity conservation, agricultural development, and rural economy revitalization.

Despite considerable experience that agricultural scientists generally have with academic research and postsecondary teaching, very few research professionals have formal training or practice in public education and outreach (Varner 2014). Yet, the success of public sector research has been linked to efficacy of public engagement (Boyer 1996), to the point where an increasing number of funding agencies require public outreach as a component of research projects (Andrews et al. 2005). As such, large-scale research efforts, and certainly any of those that are publicly funded, should include dedicated efforts to integrate public education and outreach.

Building capacity through education is essential to increasing environmentally responsible collective action (Amel et al. 2017). The topic of CWR connects important communities involved with biodiversity conservation, agriculture, and food security and as such offers many topics and curricula of potential interest to the general public. There is much to be learned from pre-existing food and agriculture literacy programs and the significant networks of individuals and organizations that can support agricultural scientists in their CWR research efforts.

This chapter focuses on the value of public education and outreach related to CWR efforts. Here, informal education is defined as any educational engagement that is not a part of formal education (i.e., not a part of k-12 or postsecondary education). Informal education compliments formal education. For example, a weekend visit to a local botanic garden can reinforce a student's recent classroom studies of habitat and food webs. Benefits and advantages of informal education include engagement of a wide audience, freedom from the formality of classroom education, the potential to engage key stakeholders such as civic leaders, and the ability to highlight subject areas, such as CWR, which are not typically covered in formal education curricula. A suite of CWR-related topics are appropriate for public education, such as the origins of domesticated plants, the value of crop relatives to agriculture, practical benefits of plant conservation, genebank conservation, plant breeding, and the utilization of genetic resources. These topics connect to a diversity of wider educational subjects including agriculture, plant conservation, food plant genetics, ethnobotany, geography, history, planning, sustainable development, and more. In practice, educational opportunities related to CWR are endless.

The goal of this chapter is to explore approaches to build CWR capacity through public education, highlight previous CWR educational programs, and identify potential stakeholders and allies for agricultural scientists working with CWR. Botanic gardens are given particular emphasis as important nodes for CWR research, outreach, and collaboration because of their strong levels of engagement with the CWR community, expertise at presenting plant science topics to the public, and their proximity to population centers in North America (Fig. 12.1).

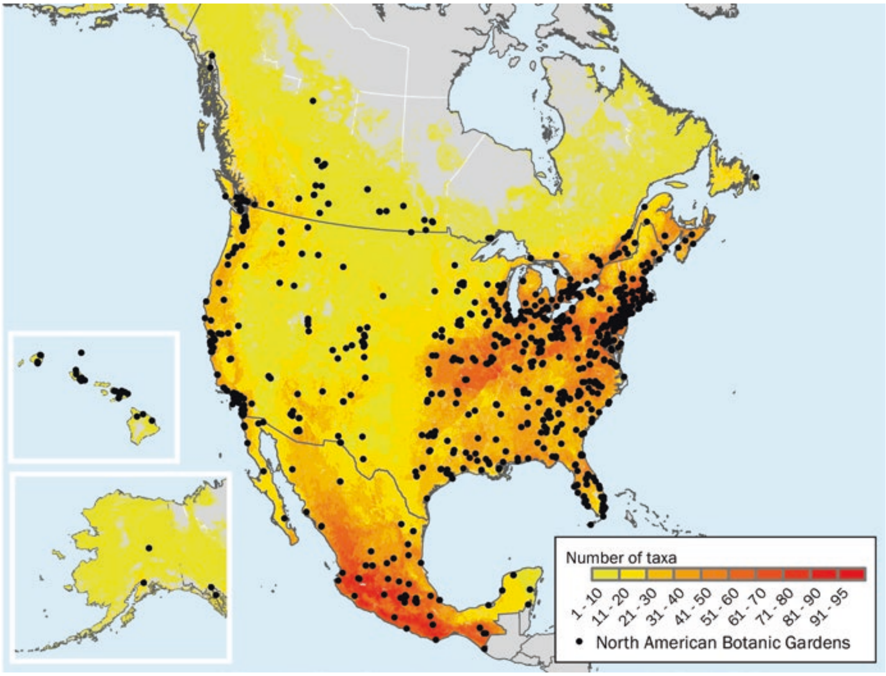

Fig. 12.1 Widespread distribution of 1037 botanical gardens in North America against a heat map of taxonomic richness of CWR (Greene et al. 2018). (Data from PlantSearch database (BGCI 2017b))

12.2 Planning and Partnering for Public Education and Outreach

12.2.1 Human Behavior, Developing Capacity, and Educational Programming

Education and outreach programs on CWR should be designed to increase the knowledge and capacity of diverse stakeholders to participate in conservation, exploration, and use, as well to increase general awareness of the subject. Audience members may be at different starting places in their understanding of biodiversity, plant conservation, agriculture, and food. Therefore, adaptable education strategies are required. While there are many different methods for public engagement in the sciences, we have outlined an adapted process combining capacity building (Hunter and Heywood 2011) and public engagement (Varner 2014) as a potential model for executing CWR education and outreach. Figure 12.2 portrays an evidence-based model for capacity building and education divided into three iterative phases: (1) development and planning, (2) implementation and action, and (3) evaluation and amplification. Development and planning involves defining goals, stakeholders, and audience while also identifying collaborators and assessing capacity assets and needs. Implementation and action involves dynamic activities (recognizing that educational scholarship has proven that dynamic approaches, such as experiential learning, are among the most effective) and formative assessment (i.e., adaptively considering the educational strategy during its execution). At this stage, reflection and feedback are very important and can be used to determine if different types of educational or training approaches can increase learning. The evaluation and

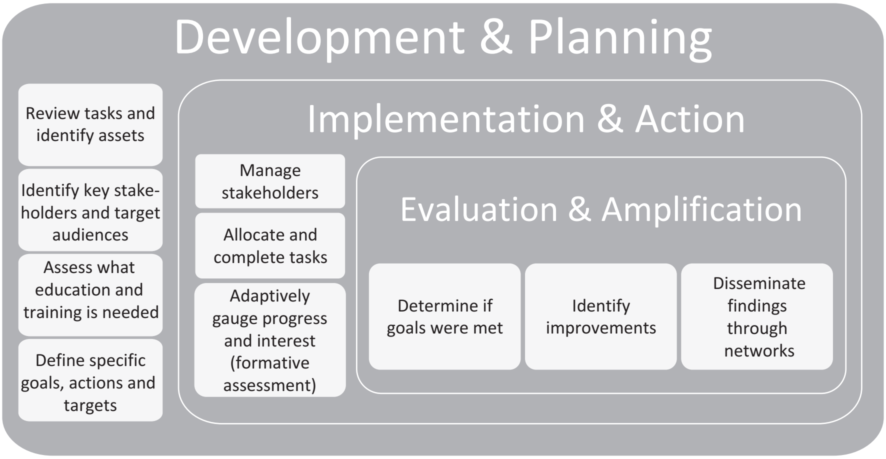

Fig. 12.2 A CWR capacity building and public engagement process. (Adapted from Varner (2014) and Hunter and Heywood (2011))

amplification phase involves evaluation, reflection, and dissemination of education and outreach results. Armed with a basic understanding of processes for public engagement, CWR researchers can design effective programs for engaging the public in their research areas.

Human behavior lies at the core of outreach, education, and conservation (Amel et al. 2017), and our evolving understanding of human behavior should be leveraged to meet outreach and education goals. Understanding drivers that impact behavior can help frame messages and create effective capacity development and educational programs (Sparkman and Walton 2017). Public engagement is impacted by factors such as personal values, attitudes, and beliefs (Schultz et al. 1995; Crompton 2010). High-impact environmentally responsible behaviors (e.g., car-free living and reduced flying) are often more difficult to change than low-impact behaviors (e.g., recycling) (Stern et al. 1999; Steg and Vlek 2009; Gifford et al. 2011). Examining factors that influence behavioral change reveals interesting insight into the power of communication and messaging. Subtle changes in messaging can have significant impacts. For example, people were twice as likely to order meatless meals when signage indicated that people were already changing their habits (now eating less meat) compared to signage that says that people are attempting to change their habits (trying to eat less meat) (Sparkman and Walton 2017). Human behavior in relation to natural resources and informal education is both well-developed, and daunting, areas of scholarship. When designing informal education programs, the researcher should seek to follow the most current knowledge on educational efficacy but will need to *right size* the level of background scholarship brought to bear in order to keep educational design tractable. As the intended outreach strategy increases in complexity, it will make increasing sense to partner with education and outreach specialists to maximize audience identification, targeting, and engagement, as well as to think though specific aspects of capacity building.

12.2.2 Educational Allies: Identifying Stakeholders Across Disciplines

A wide world of expertise in informal education and community outreach exists, often with allied scientific focus. Leveraging previous work and building effective partnerships for education and outreach can be approached in many ways depending on the capacity of the organization and the stakeholders it serves. There are no hard and fast rules for exactly who should be included as a stakeholder. However, it has been shown that creating a culture of inclusivity is essential to building equitable community food systems (Clark et al. 2017). This means welcoming people of all racial, religious, and ethnic backgrounds (especially indigenous communities who often carry the ethnobotanical knowledge of CWR), disabilities, age, sexual orientations, and gender identities. It is also important to include stakeholders from diverse political, professional, and other special interest lines. This is especially relevant in communication strategies when it comes time to hone specific messages to specific groups, as various audience subgroup attributes may necessitate unique communication tactics. Hunter and Heywood (2011) outline the following list of 13 potential CWR stakeholder groups to consider:

- Senior policy-makers and political leaders
- Biodiversity, environment, and agriculture senior decision-makers
- Leadership at relevant organizations and institutes
- Planners from local, regional, and national levels
- Scientists and researchers
- Managers of protected areas
- Project management staff
- Field technicians
- University faculty, staff, and students
- Communications and public awareness specialists
- Extension and outreach specialists
- Information analysts and managers
- Community leaders and organizations

For North American scientists working on CWR, there are a number of educational allies that have engaged around the topic and have familiarity and resources that could be used and adapted. Specifically, the Crop Science Society of America, American Society of Agronomy, Botanical Society of America, American Public Gardens Association, American Society for Plant Biology, Canadian Botanical Association, Canadian Society of Agronomy, and Mexican Association of Botanic Gardens (Asociación Mexicana de Jardines Botánicos), as well as the CGIAR network including the International Maize and Wheat Improvement Center (CIMMYT) in Mexico. In addition, the federal programs responsible for biodiversity and germplasm resources are key allies as well: La Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO) in Mexico, the US Department of Agriculture Agricultural Research Service (USDA ARS) in the USA, and both the Plant Gene Resources of Canada (PGRC) and BiodivCanada in Canada. There are also several international allies integrating CWR outreach with research programs. The largest international players in bridging the gap between CWR research and public outreach are the Millennium Seed Bank (MSB) at the Royal Botanic Gardens, Kew in Great Britain, and the Crop Trust in Bonn, Germany, who work cooperatively. The Crop Trust is intimately involved in the management of the Svalbard Global Seed Vault in Norway, and the Crop Trust's website contains a wealth of resources to relate CWR to the public (Crop Trust 2017). The Svalbard Global Seed Vault in particular has captured public attention with major media outlets at regional, national, and international levels all covering it with relatively high frequency. Quite recently, there was a front page article in The New York Times titled, "Safeguarding Seeds That May Feed the Future," about efforts to conserve agricultural diversity at the International Center for Agricultural Research in Dry Areas (ICARDA), which connected global geopolitics through the CGIAR system to Svalbard (Sengupta 2017). Additionally, international organizations such as Botanic Garden Conservation International and Bioversity International have produced excellent communications resources on CWR (BGCI 2017a; Bioversity International 2017).

Educational organizations such as the North American Association for Environmental Education, 4H, Girl Guides, Boy Scouts, Youth and United Nations Global Alliance (YUNGA), and Future Farmers of America could provide support and resources to educators who are interested in topics aligned to CWR. Agricultural extension services, which are present in many countries and active in North America, have significant knowledge of informal education and are often institutionally collocated with germplasm researchers. Additionally, organizations dedicated to the wide dissemination of applied biological science work, such as museums, botanic gardens, food and environmental activist groups, related NGOs, and even communications platforms such as TED Talks and the journalistic media, all represent potential CWR allies. In the journalism world, the excellent writing and photography by National Geographic related to food (e.g., the *Where Food Began* issue in 2008) have had tremendous public awareness impact (Mann 2008).

Environmental NGOs (ENGOs), such as the Nature Conservancy, David Suzuki Foundation, World Wildlife Fund, and many others, are involved in research, education, and outreach across North America and would be natural partners in CWR education. Regional issues and local level solutions are often raised by ENGOs with significant networks, programs, and memberships. Food policy councils (FPCs) are an important network in the USA and Canada with over 300 active councils in 2016 (Sussman and Bassarab 2016). These councils (also called food policy coalitions, collaboratives, or networks) broadly aim to support effective food policy and tend to have varying mandates and relationship/connections with different levels of government (local, regional, state/provincial, and federal). Since 2007, the number of councils in Canada and the USA has been steadily rising. This growth in food policy councils is thought to be a community response to increasing recognition of the potential role of government in addressing food system challenges, as well as the interest by food policy stakeholders to work collectively across jurisdictions and organizations (Sussman and Bassarab 2016). Many food policy councils support educational initiatives and could be important networks for raising awareness of CWR research.

We can also look to successful science outreach campaigns in North America for inspiration. The pollinator community has engaged in public outreach quite masterfully, especially since the large-scale reporting on honey bee colony collapse disorder (CCD) began in 2006. Capitalizing on this public attention, various outreach organizations related to pollinators have been successful in first educating the public that while CCD is an issue specifically of honeybees (*Apis mellifera* L.), it is but a canary in the coal mine with regard to threats across the species that provide pollinator services. These public information campaigns have been wisely linked to agriculture, both in terms of the necessity of pollinators to many crop production systems and the challenges that agriculture represents for pollinators in peril. In particular, the Million Pollinator Garden Challenge, a collaboration of many activist, science, and public engagement institutions, presents a wonderful example of a continental effort to mobilize the general public for active conservation activities and education (National Pollinator Garden Network 2017). As with CWR, an important hook of the Million Pollinator Garden Challenge is the direct link between their subject matter (pollinators) and the provisioning of food through agriculture. People certainly care about food, and with well-chosen partnerships and an eye for a dynamic learning platform, there is no reason why CWR-related groups couldn't be just as successful at capturing the public imagination as counterparts in the pollinator community.

The pollinator example is also illustrative of the successful use of citizen science, which is an excellent option for high-impact, dynamic outreach which also involves public participation in the scientific process. Citizen science has become a valuable resource for global change science, although it is still considered to be underutilized (Theobald et al. 2015). Citizens contribute to generating new scientific knowledge and understanding while simultaneously democratizing the research process. Programs take many different forms and can involve many activities, such as education, sharing of best practices, building capacity and understanding of the scientific process, and research initiatives. Public participation in research initiatives could include CWR identification, collection, conservation, planting, celebration, and advocacy. The voluntary public role in citizen science depends on many factors such as the scientific topic of study, audience, program goals and mission, and spatial and temporal ranges.

12.2.3 Partnering for Outreach: Leveraging Strengths

The most salient challenges of public outreach for the CWR research community are the lack of access to target audiences and the lack of expertise in informal educational content delivery. While all researchers would benefit from at least a cursory understanding of their intended audiences and methods of effective informal education, it is most efficient to partner with organizations and individuals specializing in these skills to deliver quality outreach. The universe of qualified and active outreach partners for CWR-related projects is immense, and it can be daunting to establish new relationships across disciplines. Tips for considering partnerships that will be mutually beneficial to all partners highlight the need to evaluate the potential for accountability, rapport, communication and understanding, realism, voice, and sustainability (Davidson and Clark 2017). Keeping these attributes in mind will help researchers identify the most fitting partners for outreach. For university-affiliated researchers, the university may have an office of public engagement and outreach that can help. In addition, faculties of communication, education, and psychology may have scholars of informal education and learning who can help to identify partners. It is also important to realize that most communities have nonprofit institutions specializing in informal education in the biological and environmental sciences that can be key points of entry into the professional world of science engagement. These include science museums, botanic gardens, and nature centers. Many scientific professional organizations also have public engagement and education staff.

12.3 Botanical Gardens for CWR Education

Botanical gardens are living museums of plants and are important nodes of CWR research, outreach, and collaboration. These gardens are located in virtually every major population center in North America. The American Public Gardens Association includes approximately 600 member institutions throughout North America, many of them botanic gardens or public gardens with science education programming. These gardens collectively welcome tens of millions of visitors annually who can benefit from the experience of learning about plants. Recent research on the geographic distribution of CWR has been built from herbarium specimens, which are overwhelmingly housed within botanic gardens where plant taxonomists assiduously describe and catalogue the diversity of plants on earth (Castañeda-Álvarez et al. 2016). As such, botanic gardens are vital learning centers about plants located among key outreach constituencies that already have strong fluency in topics related to food and agriculture.

Botanic gardens are often defined by the plants that comprise their living collections. As gardens have sought to provide greater value and relevance to society in recent decades, some have focused their collection activities on crops and CWR, even incorporating breeding programs. For example, the National Tropical Botanical Gardens in Hawaii houses the world's foremost collection of cultivated and wild breadfruit (*Artocarpus* J. R. Forst. & G. Forst. spp.) (Ragone 2007). The Minnesota Landscape Arboretum houses an impressive collection of fruit cultivars and relatives (*Malus* Mill., *Prunus* L., and *Vitis* L.) associated with its widely known breeding program (Hockenberry Meyer et al. 2010). Such gardens, in particular, are very familiar with CWR and are already engaging a general audience in outreach related to both familiar crops and their wild cousins. These institutions are excellent prospective partners both in outreach and basic CWR research.

Most public gardens see informal education as central to their mission. They often include within their staff professionals who are experts at delivering informal education to a variety of audiences and have a deep understanding of their particular community's interest in various plant-related topics. Furthermore, many public gardens are adept at both the design and subsequent evaluation of informal education activities. Recently, there have been calls from within the botanical community for gardens to engage even more deeply with agriculture and with CWR in particular (Miller et al. 2015). For example, the American Public Gardens Association recently launched a Food and Agriculture Professional Section with the specific goal to advance CWR research and outreach in North America (APGA 2017).

Like other cultural learning centers such as museums, botanic gardens create exhibits using a variety of media to relate plants to people. A major strength of botanic gardens relative to other museums is that gardens have professional horticultural staff members who can facilitate the incorporation of living plant materials into their exhibits. Many gardens are already expanding their exhibit offerings into the realm of crop and even CWR education. For example, the Louisiana State University AgCenter Botanic Gardens, which is one of many gardens associated with an agricultural university in North America, has created an exhibit titled "Corn Through the Ages," which seeks to demonstrate the history of human improvement of *Zea mays* L. from teosinte all the way through modern hybrids and genetically modified organisms (GMOs) (J. Khuehny, personal communication). The US Botanic Garden in Washington, D.C., created the exhibit "Amber Waves of Grain," which demonstrated the breeding history of wheat (Fig. 12.3), and included wheat progenitors such as einkorn, emmer, and spelt, as well as new crops such as Kernza®, which was derived from *Thinopyrum intermedium* (Host) Barkworth & D. R. Dewey, a CWR of wheat (Novy 2016). The Missouri Botanical Garden in St. Louis created an exhibit of its North American *Vitis* (grape) collection, which include CWR important as rootstocks to the commercial viticulture industry (Miller et al. 2015).

Many botanical gardens in North America are involved in food-related activities such as garden displays and exhibits, classes and lectures, training programs, and

Fig. 12.3 Wheat breeding exhibit at the US Botanic Garden engaging the public on the wonders of wheat breeding. (Photo courtesy of the US Botanic Garden)

production-based farms (Kinley 2017). At the University of British Columbia Botanical Garden (UBC-BG), the Food Garden is an important display and educational hub. The site features an outdoor classroom and new interpretive signs (including one on CWR) and is a key location used in the Sustainable Communities Field School targeting team-building activities for local businesses (Figs. 12.4 and 12.5). In addition to annual and perennial food plants, the area is also used to grow indigenous food plants such as camas (*Camassia quamash* (Pursh) Greene), wild

Food Garden

Wild Food Crop Relatives

Understanding the origins of food plants was the lifetime work of Russian scientist, Nikolai Vavilov (1887-1943).

Centres of Diversity

Vavilov is credited with associating wild crop relatives with geographic centres of food diversity and with the history of domesticating food plants. His goal was to feed the world's growing population. Vavilov's knowledge of the basic genetics of food crops was the same as what most students are taught today. However, the USSR dictated an alternate view of genetics, one with little evidence to support it. Vavilov refused to "toe the party line" and fell out of favour with the state. Imprisoned in 1940 by the USSR, he died of starvation in 1943.

Seed Security

Prior to his arrest, Vavilov led the Research Institute of Plant Industry, one of the world's first seed banks, located near Leningrad (St. Petersburg). During the 28-month Siege of Leningrad in WWII, Vavilov's colleagues took shifts keeping watch on the seed bank. Despite widespread famine in Leningrad, they refused to eat the grains, nuts, and other edible seeds stored in the Institute. By the end of the siege, nine had died of starvation.

Modern gene banks are repositories of genetic material, including seeds, used in breeding and conservation. In an attempt to avoid loss of genetic diversity, a network of gene banks has been established which now holds seed samples of over one third of the world's food crop varieties. These banks are backed by secure facilities such as Svalbard Global Seed Vault, Millennium Seed Bank, and the International Centre for Agricultural Research in Dry Areas. Today, Vavilov's Plant Industry seed bank is home to the largest collection of European fruits and berries and a partner in the world-wide network of gene banks.

Fig. 12.4 Example of crop wild relative interpretive sign developed for display at the University of British Columbia Botanical Garden. (Photo courtesy UBC Botanical Garden)

Fig. 12.5 Closing activity at UBC Botanical Garden Field School where business teams reflect on their time learning about sustainable food systems, local biodiversity, water conservation, and waste cycling in nature. (Photo courtesy UBC Botanical Garden)

strawberry (*Fragaria virginiana* Mill.), and nodding onion (*Allium cernuum* Roth). Inspired by the espalier apple collection within the Food Garden, the annual UBC Apple Festival hosts over 10,000 people during the two-day event and sells ~35,000 pounds of local apples featuring over 60 different varieties. Similarly, the Fairchild Tropical Botanic Garden in Miami celebrates its mango collection with their International Mango Festival.

Since botanic and other public gardens are already present in virtually every major population center in North America (Fig. 12.1) and have the horticultural and educational skills for excellent plant-based public education and outreach, they should be high on the list of potential collaborators for CWR outreach programs.

12.4 Future Outlook

CWR are vital genetic resources with great economic and cultural importance. Efforts to integrate public education and outreach with CWR research programs will galvanize the public's understanding of the imperative to explore, conserve, and use CWR. Drawing from psychology and climate change education research, we learn that environmental values expressed from the public can lead to legislative and infrastructural changes which in turn can reinforce additional public environmental attitudes (Tibbs 2011), which is ultimately required to achieve the public investments needed to execute robust CWR research programs. Ideally, as CWR programs continue to advance in North America, they will be accompanied by an integrated and broad effort to educate multiple segments of the public, with special emphasis on key stakeholders and decision-makers, about the societal relevance of conserving and utilizing CWR.

There is no time like the present to act. The public is indeed fascinated with the science and systems underpinning our food. There is a general sense of urgency around biodiversity conservation in general and certainly around conservation efforts integral to human well-being. Finally, research in North American crop wild relatives is active and increasing. This confluence of public interest and advancing research presents the perfect context and timing for a major public outreach and education campaign. The North American CWR community should to take advantage of this positive opportunity.

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Appendix 1
Map Methods and Occurrence Data Sources

Colin K. Khoury and Chrystian C. Sosa

The distribution maps in this book were created using occurrence (i.e., plant location/presence) information gathered from digitized herbarium records and genebank collections (i.e., passport/provenance) data, largely accessed via the Global Crop Wild Relatives Occurrence Database (CIAT 2017), Global Biodiversity Information Facility (GBIF 2017), and chapter author datasets. A full list of occurrence data providers follows this text. For taxa with few occurrence points (i.e., map coordinates) but with narrative information on location of occurrence, geo-referencing was performed via the GeoLocate web application (Tulane University 2017).

Coordinates were mapped and evaluated by chapter authors, with clearly incorrect data points deleted and additional data sought and added to the extent possible to reflect the known distributions of taxa. In some cases, available occurrence data did not sufficiently reflect the known distributions of taxa well enough for authors to be confident in their inclusion in the book; therefore, maps were not completed for these taxa.

Potential species distribution maps for taxa were modeled using the maximum entropy (Maxent) algorithm (Phillips et al. 2006), with unique occurrence locations and ecogeographic variables used as inputs. Ecogeographic variables included altitude and 19 "current" bioclimatic variables from the WorldClim database (Hijmans

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C. K. Khoury
USDA, Agricultural Research Service, Center for Agricultural Resources Research, National Laboratory for Genetic Resources Preservation, Fort Collins, CO, USA

International Center for Tropical Agriculture (CIAT), Cali, Colombia
e-mail: colin.khoury@ars.usda.gov; c.khoury@cgiar.org

C. C. Sosa
International Center for Tropical Agriculture (CIAT), Cali, Colombia

Genetics and Biotechnology Laboratory, Plant and AgriBioscience Research Centre (PABC), Ryan Institute, National University of Ireland Galway, Galway, Ireland
e-mail: c.sosaarango1@nuigalway.ie

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Table 1 Ecogeographic variables used for potential species distribution modeling

Variable number	Variable name	Units
0	Altitude	m
1	Annual mean temperature	°C
2	Mean diurnal temperature range	°C
3	Isothermality	N/A
4	Temperature seasonality (standard deviation)	°C
5	Maximum temperature of warmest month	°C
6	Minimum temperature of coldest month	°C
7	Temperature annual range	°C
8	Mean temperature of wettest quarter	°C
9	Mean temperature of driest quarter	°C
10	Mean temperature of warmest quarter	°C
11	Mean temperature of coldest quarter	°C
12	Annual precipitation	mm
13	Precipitation of wettest month	mm
14	Precipitation of driest month	mm
15	Precipitation seasonality (coefficient of variation)	%
16	Precipitation of wettest quarter	mm
17	Precipitation of driest quarter	mm
18	Precipitation of warmest quarter	mm
19	Precipitation of coldest quarter	mm
20	Bulk density	kg/m^3
21	Cation exchange capacity	cmol/kg
22	Percent clay	%
23	Organic carbon	g/kg
24	pH in H_2O	pH
25	Percent silt	%
26	Percent sand	%

et al. 2005) and seven major edaphic drivers of plant species distributions with consistent data coverage throughout North America, obtained from ISRIC-World Soil Information (Hengl et al. 2014) (Table 1).

For taxa with sufficient occurrence data (≥5 unique occurrences), a species-specific subset of the most important ecogeographic drivers of distributions was used in order to avoid overfitting (i.e., we removed highly correlated variables). To create the subsets, we used a nonlinear iterative partial least squares (NIPALS) algorithm to perform a principal component analysis (PCA), then identified those variables with the greatest contribution (>0.7 or <−0.7) to the first two principal components per taxon, and finally used a variance inflation factor (VIF) to select and use only those variables with a low degree of collinearity (Khoury et al. 2015). For taxa with fewer than five unique occurrences, all ecogeographic variables were employed.

Modeling was performed at a resolution of 2.5 arc-minutes (~5 km^2 cell size at the equator), employing 10,000 pseudo-absence background points within North America (that did not overlap with cells having presence locations) for model training, and clipped by measuring the shortest distance between the receiver operating characteristic curve (ROC curve) and the top-left corner of the plot (Liu et al. 2005). The final model was chosen among three variations: the mean and median of model replicate probabilities ($k = 10$) and the sum of thresholded areas of all model replicates, with the final choice determined by the area under the curve (AUC) (Khoury et al. 2015), true skill statistic (TSS) (Allouche et al. 2006; Georgopoulou et al. 2016), sensibility, and specificity values.

To mitigate the challenges of underfitting due to a lack of signal between occurrence points and ecogeographic variables, particularly for species with few and/or dispersed presence locations, we constrained each final model using a native range boundary defined at the US county level as given in USDA PLANTS (USDA NRCS 2017) for taxa with such information and when such information was determined by the authors and modeling team to be sufficiently comprehensive, and at the state level as given in USDA GRIN Taxonomy for Plants (USDA, ARS National Plant Germplasm System 2017) for those species not listed in USDA PLANTS or when USDA PLANTS data was not sufficiently comprehensive. For those taxa not appearing in either database, we constrained the final model with a convex hull around presence points. As a final step, models were adjusted to exclude urban areas, water bodies, bare areas, and permanent snow and ice regions (ESA GlobCover 2005). Resulting models were again evaluated by chapter authors, who made the final decision on their inclusion in the book.

The ecogeographic data preparation and species distribution modeling were performed in R (R Core Team 2013), packages maptools (Bivand and Lewin-Koh 2014), rgdal (Bivand et al. 2014), SDMTools (van der Wal et al. 2014), raster (Hijmans 2014), sp (Pebesma and Bivand 2005; Bivand et al. 2013), dismo (Hijmans et al. 2013), plsdepot (Sanchez 2012), and usdm (Naimi 2015). Resulting spatial files were mapped in ArcMap (ESRI 2011) and in R packages ggmap (Kahle and Wickham 2013; Kahle and Wickham 2016) and ggplot2 (Wickham and Chang 2016).

For access to the occurrence data used in this book, as well as potential distribution models for individual taxa, please contact the editors.

List of Occurrence Data Providers

AAU (Aarhus University); ACAD (Philadelphia Herbarium at the Academy of Natural Sciences); Acadia (Acadia University); AGG (Australian Grains Genebank); Agriculture and Agri-Food Canada (Canadian Biodiversity Information Facility); ALA (University of Alaska Fairbanks Herbarium); Ales Lebeda; ALTA-VP (University of Alberta Vascular Plant Herbarium); Anymals.org (Museum für Naturkunde Berlin); ARIZ (University of Arizona Herbarium); ASC Deaver Herbarium (Northern Arizona University); ASU (Arizona State University Global Institute for Sustainability); AVH (Australian Virtual Herbarium); AVRDC (World Vegetable Center); AWC (Arizona Western College Herbarium); BAYRT (via BIEN); BC (Royal BC Museum); BCMEX (University of Baja California Herbarium); BDI (Putnam Museum herbarium); BGBM (Botanic Garden and

Botanical Museum Berlin-Dahlem); Biodiversity Institute of Ontario; Bioimages; Bioversity International; BISON (United States Geological Survey); BLM (Bureau of Land Management); BM (British Museum of Natural History); BNHM-UCB (Berkeley Natural History Museum); BOUM (Muséum d'Histoire Naturelle de Bourges); BPBM (Bernice Pauahi Bishop Museum); BRIT (Botanical Research Institute of Texas); BRY (Brigham Young University Herbarium); BU (Brock University); BUT (Butler University Friesner Herbarium); California State University Chico; CAN (National Herbarium of Canada); CANB (National Herbarium of New South Wales); CAS (California Academy of Sciences); CATIE (Centro Agronómico Tropical de Investigación y Enseñanza); CCH (Consortium of California Herbaria); CDA (California Department of Food and Agriculture Herbarium); CEN (EMBRAPA Recursos Geneticos e Biotecnologia – CENARGEN Herbario); CEPLAC (Comissão Executiva do Plano da Lavoura Cacaueira); CERETI (via BIEN); CHAP (Universidad Autonoma Chapingo Herbario); CHIC (University of Illinois Herbarium); CHR (Landcare Research); CHSC (the Chico State Herbarium); CIAT (International Center for Tropical Agriculture a global database for the distributions of crop wild relatives); CIB (Universidad Veracruzana Herbario); CIBNOR (Centro de Investigaciones Biológicas del Noroeste Herbario); CIB-UAEH (Centro de Investigaciones Biológicas Universidad Autónoma del Estado de Hidalgo); CIB-UV (Centro de Investigaciones Biológicas Universidad Veracruzana); CIBYC-UAEM (Centro de Investigación en Biodiversidad y Conservación Universidad Autónoma del Estado de Morelos); CICB-UAT (Centro de Investigación en Ciencias Biológicas de la Universidad Autónoma de Tlaxcala); CICY (Centro de Investigacion Cientifica de Yucatan A.C. Herbario); CIHS-UAC (Universidad Autónoma de Campeche); CIIDIR-DURANGO/CIIDIR-IPN-DGO (Instituto Politecnico Nacional Herbario Durango); CIIDIR-IPN (Instituto Politecnico Nacional Herbario); CIMI (Centro Interdisciplinario de Investigacion para el Desarrollo Integral Regional Michoacan Herbario); CIMMYT (International Center for the Improvement of Maize and Wheat); CIP (International Potato Center); Claire Heinitz; CLARK-A (Clark Atlanta University); CLEMS (Clemson University Herbarium); CM (Carnegie Museums); CMN (Canadian Museum of Nature); CNH (Consortium of Northeastern Herbaria); CNS-UT (College of Natural Sciences University of Texas at Austin); COA (Botanical Garden of Córdoba); COAH (Instituto Amazonico de Investigaciones Cientificas SINCHI Herbario Amazonico Colombiano); COL (Universidad Nacional de Colombia); COLO (University of Colorado Museum Herbarium); CONABIO (La Comisión Nacional para el Conocimiento y Uso de la Biodiversidad); CONN (University of Connecticut Herbarium); Consortium of California Herbaria; Coppens; COTECOCA (Comisión Técnico Consultiva de Coeficientes de Agostadero); CP (Faculty of Life Sciences University of Copenhagen Herbarium Botany Group); CP-CT (via BIEN); CPNWH (Consortium of Pacific Northwest Herbaria); CRIA (Centro de Referência em Informação Ambiental); CS (Colorado State University Herbarium); CUVC (Universidad del Valle Herbario Luis Sigifredo Espinal-Tascon); CZE National PGR Inventory; DACB-UJAT (División Académica de Ciencias Basicas Universidad Juárez Autónoma de Tabasco); DAV (University of California Davis); DBG (Denver Botanic Gardens); DES (Desert Botanical Garden Herbarium); DICTUS-USON

(Departamento de Investigaciones Científicas y Tecnológicas de la Universidad de Sonora); DS (California Academy of Sciences Dudley Herbarium); DSC (Delta State University Herbarium); DSUND (Dickinson State University Herbarium); E (Royal Botanic Garden Edinburgh); EB-BUAP (Escuela de Biología Benemérita Universidad Autónoma de Puebla); ECOSUR (El Colegio de la Frontera Sur); ECPGR (European Cooperative Programme for Plant Genetic Resources); EIU (Eastern Illinois University Stover-Ebinger Herbarium); EKY (Eastern Kentucky University Herbarium); EMBL-EBI (European Bioinformatics Institute); EMC (Eastern Michigan University Herbarium); ENCB (Instituto Politecnico Nacional Herbario Mexico); ENLC (Eastern Nevada Landscape Coalition); ENMU (Eastern New Mexico University Herbarium); EST (via BIEN); EURISCO (European Search Catalogue for Plant Genetic Resources); F (Field Museum); FB-UMSNH (La Universidad Michoacana de San Nicolás de Hidalgo); FCB-UAEM (Facultad de Ciencias Biológicas Universidad Autónoma del Estado de Morelos); FCF-UANL (Facultad de Ciencias Forestales Universidad Autónoma de Nuevo León); FCN-UAQ (Facultad de Ciencias Naturales Universidad Autónoma de Querétaro); Universidad Nacional de la Plata; FC-UABC (Facultad de Ciencias Universidad Autónoma de Baja California); FC-UNAM (Universidad Nacional Autónoma de México); Fernando de la Torre; FESI-UNAM (Universidad Nacional Autónoma de México); FFPRI (National Institute of Genetics ROIS); FITECMA-UMSNH (Universidad Michoacana de San Nicolás de Hidalgo); FLAS (Florida Museum of Natural History); Flora Nacional Cumbres Monterrey; FML (Fundacion Miguel Lillo); FMVZ-UADY (Campus de Ciencias Biológicas y Agropecuarias Universidad Autónoma de Yucatán); FR (Senckenberg); FRT (via BIEN); FSU (Florida State University Herbarium); FTG (Fairchild Tropical Botanic Garden); G (Conservatoire et Jardin botaniques de la Ville de Genève); GA (University of Georgia Herbarium); GB (University of Gothenburg Herbarium); GBIF (Global Biodiversity Information Facility); GCNP (Grand Canyon National Park Herbarium); GDA (Universidad de Granada Herbario); GEO (Tag der Artenvielfalt); GMDRC (Granite Mountains Desert Research Center Herbarium); GOET (Georg-August-Universität Göttingen Albrecht-von-Haller-Institut für Pflanzenwissenschaften Abteilung Systematische Botanik); Gouvernement du Quebec; Greater Good; GUA (DIVEA, DEP, FEEMA Herbario Alberto Castellanos Rio de Janeiro Brazil); GZU (University of Graz Institute of Plant Sciences); H (Finnish Museum of Natural History); H.A. Stephens Herbarium; HAL (Martin-Luther-Universitet Herbarium); HAM (Royal Botanical Gardens Herbarium Canada); HUH (Harvard University Herbaria); HAST (Biodiversity Research Center Academia Sinica Herbarium Taiwan); HBC (Herbarium Bradeanum Herbario Brazil); HBG (Biozentrum Klein-Flottbek Herbarium Germany); HCIB (Centro de Investigaciones Biologicas del Noroeste S. C. Herbario Mexico; Herbarium GeoBHinton; HIBG (High Plains Herbarium at Chadron State College); HNT (Huntington Botanical Gardens Herbarium); HPC (Howard Payne University Herbarium); HSC (Humboldt State University Vascular Plant Herbarium); HU (University of Zhejiang Herbarium); IAC (Instituto Agronômico); IB-CUCBA-UDG (Centro Universitario de Ciencias Biológicas y Agropecuaria Universidad de Guadalajara); IBT (Instituto de Botânica); IBUG (Universidad de Guadalajara Herbario); IBUNAM (Arboles de la Península de

Yucatán/ Flora del Distrito de Tehuantepec/Oaxaca y Familia Asteraceae en México); ICBG (Agentes Bioactivos de Plantas Desérticas de Latinoamérica); IE (Comisión nacional para el conocimiento y uso de la biodiversidad); IEA-UAT (Instituto de Ecologia Aplicada Universidad Autónoma de Tamaulipas); IEB (Instituto de Ecologica A.C. Herbario del Centro Regional del Bajio); IE-DF/ UNAM (Arboles y Arbustos Nativos para la Restauración Ecológica y Reforestación de México); IEX (via BIEN); IF (Instituto Florestal); IHNE (Instituto de Historia Natural y Ecología Chiapas Mexico); IIZD-UASLP (Instituto de Investigación de Zonas Desérticas Universidad Autónoma de San Luis Potosí); ILLS (Illinois Natural History Survey Herbarium); IMC y P (via BIEN); IMECBIO-UDG (Departamento de Ecología y Recursos Naturales Universidad de Guadalajara); iNaturalist; INECOL (Instituto de Ecología A.C.); INHS (Illinois Natural History Survey); INIFAP/ INIFAP-CECOY/INIFAP-SARH/INIFAP-UPN (Instituto Nacional de Investigaciones Forestales y Agropecuarias Mexico); INIREB (Instituto Nacional de Investigaciones En Recursos Bióticos Mexico); INPA (Instituto Nacional de Pesquisas da Amazônia); IPA (Instituto Agronômico de Pernambuco); IPN (Instituto Politécnico Nacional); IRENAT-CP (Instituo de Rescursos Naturales Mexico); IRN-CP (via BIEN); IRVC (Irvine Herbarium); JABOT (Jardim Botânico do Rio de Janeiro); James Frelichowski; Jardin Botanique de Montréal; JBRJ (Rio de Janeiro Botanical Garden Herbarium Collection); JEMEZ (Jemez Mountain Herbarium); JEO (via BIEN); JOTR (Joshua Tree National Park Herbarium); JROH (Oakmead Herbarium and Collections of Jasper Ridge Biological Preserve); K (Royal Botanic Gardens Kew); K MSB (Royal Botanic Gardens Kew Millennium Seed Bank Partnership); KANU (University of Kansas R. L. McGregor Herbarium); Karen Williams; KHD (Kathryn Kalmbach Herbarium); Kim Hummer; KNK (Northern Kentucky University Herbarium); KNWR (Arctos); KSP (Pittsburg State University Theodore M. Sperry Herbarium); KSTC (Emporia State University); KU (University of Kansas Biodiversity Institute); LA (University of California Herbarium Los Angeles); Laura Marek; LD (Lund Botanical Museum); LEGON-GC (Ghana Biodiversity Information Facility (GhaBIF)); LISC (Instituto de Investigação Científica Tropical Portugal); LISU (National Museum of Natural History and Science University of Lisbon); LL (University of Texas at Austin Lundell Herbarium); LSU (Louisiana State University Herbarium); M (Botanische Staatssammlung Munchen Herbarium); MA (CSIC-Real Jardín Botánico); MABA (New Mexico Natural History Institute Herbarium); MACN (Museo Argentino de Ciencias Naturales); MADUG; MANCH (University of Manchester Herbarium); MARY (University of Maryland Norton-Brown Herbarium); MB (Philipps University Marburg Herbarium Marburgense); McDonald & Austin 1990; Melanie Harrison; MESA (Walter Almond Kelley Herbarium); Mexico Burge; MexSEINet; MEXU (Universidad Nacional Autonoma de Mexico Herbario Nacional); MICH (University of Michigan Herbarium); Midwest Herbaria; MIREN_ETH (Mountain Invasion Research Network); MISS (University of Mississippi Pullen Herbarium); MISSA (Mississippi Entomological Museum); MISU (Minot State University Herbarium); MMNS (Mississippi Museum of Natural Science Herbarium); MNA (Museum of Northern Arizona); MNCH (University of Oregon Museum of Natural and Cultural History); MNCR (Museo Nacional de Costa Rica); MNHN (Museum national d'Histoire naturelle); MO (Missouri Botanical Garden); MOR (Morton Arboretum Herbarium); MPM (Milwaukee Public Museum); MSC (Michigan State University Herbarium); MT (Universite de Montreal Herbier Marie-Victorin); MTTHORT (via BIEN); MUR (Murray State University Herbarium); MWI (R.M. Myers Herbarium); NA (United States National Arboretum USDA/ARS Herbarium); Naturalis (Naturalis Biodiversity Center); Naturgucker (naturgucker.de); NCSC (North Carolina State University Herbarium); NCU (University of North Carolina Herbarium); ND (University of Notre Dame Greene/Nieuwland Herbarium); NHM UK (Natural History Museum UK); NHN Leiden (National Herbarium of the Netherlands); NMC (New Mexico State University Herbarium); NMCR (New Mexico State University Range Science Herbarium); NMSU (Northwest Missouri State University Herbarium); NordGen (Nordic Genetic Resource Center); NPS (Inventory and Monitoring Program – NPSpecies Park Species Lists); NSW (the Royal Botanic Gardens and Domain Trust); NTSRV (NatureServe); NYBG (New York Botanic Garden); O (Natural History Museum University of Oslo); OAC (University of Guelph OAC Herbarium); OBI (Cal Poly State University Herbarium); OHN (Biological Museum, Oskarshamn Herbarium); OKLA (Oklahoma State University Herbarium); OSA (National Museum of Nature and Science Japan); OSC (Oregon State University); OTS (Organization for Tropical Studies Herbarium); Pablo Jourdan; PARADA; PBDB (Paleobiology Database); PH (Academy of Natural Sciences Herbarium Philadelphia); Plants of Taiwan; PMT (via BIEN); POM (Pomona College Herbarium); Programa de repatriacion de datos de ejemplares mexicanos; QFA (Universite Laval Herbier Louis-Marie); QMEX (Universidad Autonoma de Queretaro Centro Universitario Herbario); R. L. McGregor Herbarium Vascular Plants Collection; Reilley; RENO (University of Nevada Herbarium); RM (University of Wyoming Rocky Mountain Herbarium); RMBL (Rocky Mountain Biological Laboratory); RMCA-Metafro-Infosys (via BIEN); ROM (Royal Ontario Museum (ROM)); Rosalinda Gonzalez Santos; RSA/RSABG (Rancho Santa Ana Botanic Garden Herbarium); SALA (Dep. of Plant Biology Botany Faculty of Pharmacy Univ. Salamanca); SANBI (South African National Biodiversity Institute); SANT (Herbario SANT Universidade de Santiago de Compostela); SBBG (Santa Barbara Botanic Garden Herbarium); SCFS (Sagehen Herbarium); SCIR (Santa Cruz Island Reserve Herbarium); SD/SDNHM (San Diego Natural History Museum Herbarium); SDSU (San Diego State University); SEINET (Regional Networks of North American Herbaria); SERBO AC (via BIEN); SEV (Department of Plant Biology and Ecology University of Seville); SFV (California State University Northridge); SI (Museo Botanico Herbarium Argentina); SINGER (System-Wide Information Network on Genetic Resources); SJNM (San Juan College Herbarium); SJSU (Carl W. Sharsmith Herbarium San Jose State University); SMNK (State Museum of Natural History Karlsruhe); SNM (Western New Mexico University Dale A. Zimmerman Herbarium); SNSNMC (Société des Sciences Naturelles et Mathématiques de Cherbourg); Sonoran Atlas; Stebbins 2013; STU (Staatliches Museum fur Naturkunde Herbarium); SUU (Southern Utah University's Herbarium); Swain 2012; TAIF (Taiwan Forestry Research Institute); TAMU (Texas A&M University Herbarium); TEX (University of Texas at Austin Herbarium); TLAX (Universidad

Autónoma de Tlaxcala); TLMF (Tiroler Landesmuseum Ferdinandeum); TRH (Norwegian University of Science and Technology Herbarium); TROM (UiT The Arctic University of Norway); Tropicos; TROY (Troy University Herbarium); TTRS (Tall Timbers Research Station Herbarium); U (National Herbarium of the Netherlands Herbarium Utrecht); UA (University of Alabama Biodiversity and Systematics); UAAAN (Universidad Autonoma Agraria Antonio Narro); UABC (Universidad Autonoma de Baja California); UACh (Universidad Autónoma de Chihuahua); UAG (University of Guadalajara); UAM (University of Alaska Museum of the North); UAM (University of Arkansas at Monticello Herbarium); UAN (Universidad Autónoma de Nayarit); UANL (Universidad Autónoma de Nuevo León); UAQ (Universidad Autónoma de Querétaro); UAS (Universidad Autonoma de Sinaloa Herbario Jesus Gonzalez Ortega); UBC (University of British Columbia Herbarium – Vascular Plant Collection); UC/JEPS (University of California Jepson Herbarium); UCB (University and Jepson Herbaria); UCBG (University of Botswana Herbarium); UCD (University of California Davis); UCMC (University of Colorado Museum of Natural History); UCMP (University of California Museum of Paleontology); UConn (University of Connecticut); UCR (University of California Riverside); UCS (Universidade de Caxias do Sul); UCSB (University of California Santa Barbara; UCSC (University of California Santa Cruz); UEFS (Universidade Estadual de Feira de Santana); UFMG (Universidade Federal de Minas Gerais); UFPR (Universidade Federal do Paraná); UFRGS (Universidade Federal do Rio Grande do Sul); UFSC (Universidade Federal de Santa Catarina); Uglasiirsity; UM (Herbarium of Université de Montpellier 2 Institut de Botanique); UMN (University of Minnesota Herbarium); UNA (University of Alabama Herbarium); UNAM (National Autonomous University of Mexico); UNESP/FCA (Universidade Estadual Paulista); UNICACH (Universidad de Ciencias y Artes de Chiapas); UNICAMP (Universidade Estadual de Campinas – Instituto de Biologia); Universidad de Antioquia; Universite de Montreal Biodiversity Centre; Université Laval; University of Alberta Museums; University of Arizona; University of Lethbridge; University of Manitoba; UNM (Museum of Southwestern Biology); UPS (Uppsala University Herbarium); US (National Museum of Natural History Smithsonian Institution); USCH (University of South Carolina Herbarium); USDA NPGS GRIN (USDA National Plant Germplasm System Genetic Resources Information Network); USDA PLANTS Database; USF (USF Water Institute); USFS (United States Forest Service); USMS (University of Southern Mississippi Herbarium); USNM (Smithsonian); USON (Universidad de Sonora Herbario); USP (Universidade de São Paulo); USU (Utah State University); USUUB (Utah State University Uintah Basin Herbarium); UT (University of Utah Garrett Herbarium); UTC (Utah State University Intermountain Herbarium); UTEP (University of Texas at El Paso Biodiversity Collections); UVSC (Utah Valley University Herbarium); UWBM (University of Washington Burke Museum); UWSA (University of Wisconsin); UWSP (University of Wisconsin Stevens Point); VAL (University of Valencia); VegBank – Vegetation Plot Database; VIT (Natural History Museum of Alava (Museo de Ciencias Naturales de Álava)); VSC (Valdosta State University Herbarium); W (Natural History Museum Vienna Herbarium);

WAG (Wageningen University National Herbarium Nederland Wageningen University branch); WASH; WCUH (Western Carolina University Herbarium); Wildlife Sightings; WILLI (Herbarium of the College of William & Mary); Wilson et al. 2017; WIS (University of Wisconsin Herbarium); WOODS; WTU (University of Washington Herbarium); WU (Universitat Wien Herbarium); WVU (West Virginia University); YM-YOSE (Yosemite National Park Herbarium); YPM (Yale University Peabody Museum); Z (Herbaria of the University and ETH Zürich (Z + ZT)); ZSS (Sukkulenten-Sammlung Zurich Herbarium).

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Common Name Index

A
Acorn squash, 98
African oil palm, 174
Agave, 7, 70, 73, 75–78, 119, 120, 130, 258
Alfalfa, 179, 301, 303
Amaranth, 7, 64, 70, 72, 73, 76–78, 98, 112, 115
American black currant, 105
American Chestnut, 123
American cotton, 5
American cranberry, 98
American ginseng, 5, 105, 131
American gooseberry, 98
American persimmon, 112, 124
American red raspberry, 104
Annatto, 70, 76
Annual phlox, 5, 9
Apple, 112, 115, 118, 203, 264, 322
Arizona black walnut, 127
Avocado, 6, 9, 64, 70, 72, 73, 76, 77, 82, 113
Awned wheatgrass, 47

B
Bakersfield prickly pear cactus, 64
Barley, 6, 40, 43, 50, 98, 112, 113, 118, 179, 222
Beach strawberry, 102
Bean, 6, 11, 64, 70, 72, 73, 76, 77, 80–81, 86, 98, 99, 112, 123, 129, 203
Beet, 112, 121, 214
Begonia, 119
Big blue stem, 7, 126
Blackberry, 9, 15, 44, 104, 112
Black cohosh, 105, 131
Black currant, 104
Black persimmon, 112, 124
Black raspberry, 9, 104, 112
Black sapote, 8
Bladderpod, 105, 113
Blueberry, 10, 15, 18, 39, 44, 98, 99, 101, 112, 123
Bluebunch wheatgrass, 299
Bluegrass, 299
Blue honeysuckle, 39, 47
Breadfruit, 113, 319
Brinjal eggplant, 178
Bromeliad, 70, 75, 76
Bushy bluestem, 7, 47
Butternut, 105, 124
Butternut squash, 100

C
Cacao, 6, 10, 15, 65, 70, 73, 76, 77, 113
Cactus, 11, 64, 70, 73, 75, 76, 87
California black currant, 104
Canadian milkvetch, 47
Carrot, 112, 115, 179, 299, 301, 302
Cassava, 70, 73, 76, 129, 179
Celery, 302
Chayote, 10, 64, 70, 76, 77
Chenopod, 7, 98
Cherimoya, 7, 73
Cherry, 65, 77, 104, 112, 115, 124
Chestnut, 112, 125
Chia, 9
Chile pepper, 81, 102
Chrysanthemum, 259
Cone flower, 8
Cotton, 5, 6, 8, 64, 70, 73, 76, 78, 81, 85, 98, 105, 112, 115, 118, 124, 137, 179, 203

S. L. Greene et al. (eds.), *North American Crop Wild Relatives, Volume 1*,
https://doi.org/10.1007/978-3-319-95101-0

Cow cockle, 44, 54
Cranberry, 10, 15, 39, 44, 47, 53, 98, 99, 101–102, 123, 137, 140, 148, 160
Currant, 13, 15, 44, 47, 104, 123, 129
Cushaw, 8, 124

D
Dahlia, 70, 73, 75, 76
Date palm, 174
Devilys claw, 6, 9, 98
Dill, 302
Dragon fruit, 70, 73, 75–77, 302

E
Eastern black walnut, 127
Eastern gamagrass, 126
Echeveria, 70, 73, 75, 76
Eggplant, 129, 175, 177–179, 183, 184
Elderberry, 44
English walnut, 104
Erect knotwood, 98
European black currant, 104
European gooseberry, 104

F
Fanweed, 49

G
Goatnut, 70
Golden eagle, 168
Goldenseal, 105, 131, 157
Gooseberry, 15, 44, 104, 129
Gourds, 137
Grape, 15, 70, 73, 76, 98–100, 102, 112, 115, 119, 125, 132, 320
Grass, 7, 8, 10, 44, 45, 52, 53, 98, 110, 118, 126
Guava, 9, 70, 76, 113, 123
Guayule, 5, 9, 105, 113

H
Hawaiian strawberry, 194
Hibiscus, 120, 157, 260
Hickory, 112
Highbush blueberry, 10, 101
Highbush cranberry, 47
Hops, 5, 46, 98
Husk tomato, 9, 72, 73, 76, 77, 86

J
Jerusalem artichoke, 8, 37
Jicama, 8
Jojoba, 5, 72, 113
Juneberry, 39

K
Kale, 302

L
Large-fruited cranberry, 101
Lesquerella, 105
Lettuce, 112, 115, 123
Liatris, 157
Lima bean, 11, 81
Lingonberries, 123
Little barley, 6, 98
Little bluestem, 47
Lowbush blueberry, 101

M
Maize, 5, 6, 10, 11, 15, 37, 70, 72, 73, 75–80, 84–86, 89, 98, 112, 113, 115, 119, 123, 158, 179, 203, 303
Marigold, 65, 73, 76
Marmalade-plum, 73
Marshelder, 98
Maygrass, 98
Meadowfoam, 105, 113
Mexican hawthorn, 70, 73, 76
Millet, 112, 115
Monarda, 44, 51
Muscadine grape, 98, 102

N
Nance, 70, 73, 76
Nodding onion, 322
Nopal, 64, 70, 75–77
North American black raspberry, 9
North American gooseberry, 104
Northern California black walnut, 105
Northern highbush blueberry, 101
Northern wheatgrass, 47

O
Oat, 40, 43, 44, 50, 112
Oilseed brassica, 112, 302
Oilseed rape, 301

Onion, 112, 115, 129, 301, 302, 322
Orchid, 45, 70, 73, 75, 76, 178

P
Papaya, 7, 20, 70, 73, 76, 113
Pawpaw, 7, 98, 103–104, 127
Peach, 104, 115, 124
Pecan, 7, 11, 18, 70, 76, 98, 99, 101, 112, 115, 123, 125, 127
Pecan hickory, 99, 112
Pennycress, 49
Penstemon, 261, 283, 297
Pepo squash, 100
Pepper, 64, 72, 73, 76, 77, 81, 98, 99, 112, 120, 124, 175
Persimmon, 112, 124
Phlox, 5, 9, 119, 261
Physic nut, 70, 75, 76
Pistachio, 112, 115, 124
Pitaya, 70, 76
Plains bristle grass, 34
Plains rough fescue, 47
Plum, 10, 18, 104, 115, 124, 126, 129
Poinsettia, 75, 76
Ponytail palm, 70, 73, 75, 76
Portulaca, 44, 70, 76, 261
Potato, 43, 64, 72, 76, 77, 81, 86, 98, 112, 115, 120, 124, 129, 148, 175, 179, 203
Prickly pear, 8, 64, 113
Pumpkin, 6, 8, 11, 77, 78, 98–100, 115, 124, 129
Purple mombin, 10, 70, 75, 76
Purple prairie clover, 44, 47
Purple raspberries, 104
Purslane, 44, 74, 76

Q
Quelite, 70, 76
Quinoa, 98, 99, 115

R
Radish, 302
Ramps, 123
Raspberry, 15, 44, 47, 98, 104, 112, 115, 124
Red currant, 104
Red raspberry, 104
Rice, 78, 112, 113, 161, 173, 179, 269, 303
Rock grape, 132
Rose, 46, 75, 76, 85, 121, 157, 300
Rudbeckia, 5, 119, 262
Runner bean, 9, 11, 73, 81
Ryegrass, 299

S
Salvia, 9, 119, 120, 129
Sand blue stem, 126
Sapotes, 70, 76
Saskatoon berry, 39, 46
Seepweed, 70, 76
Silene, 180
Small cranberry, 52, 102, 137, 140, 160
Snapdragon, 302
Sonoran panic grass, 98
Sorghum, 14, 112, 120, 179, 246, 262
Southern highbush blueberry, 101
Soybean, 112, 179
Sparsley, 34
Squash, 6, 8, 11, 37, 64, 70, 73, 76, 98–100, 112, 115, 124, 129
Stinkweed, 49
Strawberry, 8, 15, 46, 99, 101, 102, 112, 115, 124, 322
Sugar beet, 112, 121
Sugarcane, 113, 179
Sunflower, 5, 8, 11, 13, 15, 18, 19, 37, 50, 51, 70, 72, 76, 77, 98–101, 112, 115, 121, 124, 129, 146, 148, 179, 203, 240, 284
Sweet peppers, 301
Sweet potato, 65, 70, 73, 76, 77, 99, 112, 115, 121, 124, 146, 171, 172, 179, 203, 217
Switch grass, 9, 126

T
Teosinte, 65, 73, 77–80, 84, 297, 320
Tepary bean, 9, 81, 98, 137
Tequila agave, 77
Texas wildrice, 126
Tobacco, 37, 121, 175
Tomatillo, 9
Tomato, 64, 76–78, 81, 112, 113, 129, 175, 182
Trillium, 130
Turnip, 301

V
Vanilla, 5, 6, 10, 64, 70, 75–77, 125, 262
Viola, 119
Virginia strawberry, 102

W
Walnut, 104–105, 112, 115, 125, 127, 132
Western wheatgrass, 47
Wheat, 40, 43, 79, 112, 113, 169, 173, 179, 222, 283, 284, 320
Wheat grass, 47, 126, 299
Wild northern highbush blueberry, 101
Wildrice, 98, 100, 103, 115, 125–127, 129
Wild rye, 126
Wild strawberry, 321–322
Winterfat, 47

Y
Yam bean, 8
Year bean, 81
Yucca, 119

Scientific Name Index

A
Abutilon, 258
Acer spp., 258
  A. saccharum, 124
Actaea spp., 258
  A. racemosa, 105, 131
Aegilops spp., 44, 112, 258
  A. cylindrica, 42
Agave spp., 7, 70, 73, 75–78, 119, 120, 130, 258
Agavaceae, 74
Agave spp., 7, 70, 73, 75–78, 119, 120, 130, 258
  A. angustifolia, 78
  A. tequilana, 7
Agropyron spp., 258
Agrostis, 105, 258
Alliacea, 74
Allium spp., 13, 14, 120, 123, 322
  A. cernuum, 322
  A. fistulosum, 123
  A. munzii, 129, 132
  A. porrum, 123
  A. sativum, 123
  A. tricoccum, 123
Alopecurus, 258
Amaranthaceae, 74
Amaranthus spp., 50, 64, 70, 76, 88, 120, 129, 258, 301
  A. hybridus, 98
  A. caudatus, 7
Amaryllidaceae, 74, 123
Amelanchier alnifolia, 39
Anacardiacea, 74, 124
Ananas, 65
Andropogon spp., 118, 258
  A. gerardi, 7, 126
  A. gerardii, 7
Annonaceae, 74
Annona spp., 70, 258
  A. cherimola, 7
Apiaceae, 74, 263, 302
Apios spp., 7, 258
  A. americana, 7
Apis mellifera, 301, 317
Apium, 258
Apocynaceae, 74
Aquila chrysaetos, 168
Armoracia, 13, 14, 258
Aronia, 258
Arrhenatherum, 258
Artemisia spp., 258, 274
Artocarpus, 14, 258, 319
Asclepias, 119, 120
Asimina spp., 258
  A. triloba, 7, 103, 127
Asparagaceae, 74
Asparagus, 14, 258
Asteraceae, 74, 78, 123, 124, 174, 263, 303, 304
Astragalus spp., 105, 118, 302
  A. canadensis, 47
Atriplex, 129, 258
Avena spp., 14, 50, 258
  A. fatua, 42, 50

B
Bassia, 258
Beaucarnea, 70, 73, 75, 76
Begonia, 119
Begoniaceae, 74

S. L. Greene et al. (eds.), *North American Crop Wild Relatives, Volume 1*,
https://doi.org/10.1007/978-3-319-95101-0

Beta spp., 14, 121, 258
  B. vulgaris, 121
  B. vulgaris subsp. *maritima,* 121
Betula, 121
Bixa, 70, 76
Bixaceae, 74
Blitum, 119
Boehmeria, 258
Boraginaceae, 74
Bouteloua, 7, 118
Brassicaceae, 54, 74
Brassica spp., 14, 112, 118, 258, 302
  B. napus, 301
  B. rapa, 301
Bromeliaceae, 74
Bromus spp., 45, 50, 105, 258
  B. inermis, 303
Byrsonima spp., 70, 76
  B. crassifolia, 73

C
Cactaceae, 70, 74–76, 78
Camas, 321
Camassia quamash, 321
Camelina spp., 50
  C. sativa, 46, 50
Canavalia, 258
Capparis, 258
Capsella bursa-pastoris, 50
Capsicum spp., 14, 64, 76, 81, 88, 99, 120, 258
  C. annuum, 6, 15
  C. annuum var. *glabriusculum,* 137, 203
  C. baccatum var. *pendulum,* 124
Caricaceae, 74
Carica spp., 88, 121, 258
  C. papaya, 7, 20, 70, 76
Carthamus, 14, 129, 258
Carya spp., 118, 258
  C. floridana, 118
  C. glabra, 118
  C. glabra var. *odorata,* 118
  C. illinoinensis, 7, 99, 101, 123
Casimiroa edulis, 7
Castanea spp., 121, 258
  C. mollissima, 123
Ceanothus, 119
Cercis, 130
Chamaecyparis, 117
Chenopodiaceae, 74, 263
Chenopodium spp., 297
  C. berlandieri, 15, 16, 98, 288
  C. berlandieri subsp. *nuttalliae,* 7
  C. neomexicanum, 288
  C. quinoa, 99, 284, 288
  C. standleyanum, 289
  C. watsonii, 289
Chrysanthemum, 259
Cinnamomum, 259
Cleomaceae, 74
Cochlearia, 259
Cocos spp., 259
  C. nucifera, 174
Coffea, 246
Coix, 259
Colocasia, 14, 259
Convolvulaceae, 74, 124
Corchorus, 259
Corallorhiza striata, 178
Coreopsis spp., 119, 259
  C. palmata, 118
  C. tinctoria, 259
Cornus, 121, 130
Corylus spp., 13, 14, 121, 259
  C. americana, 121
  C. avellana, 123
  C. californica, 121
Cotinus obovatus, 121
Crassulaceae, 75
Crataegus, 70, 76, 259
Crotalaria, 120, 259
Croton spp., 259
Cucumis, 14, 259
Cucurbitaceae, 75, 124
Cucurbita spp., 14, 64, 70, 76, 88, 99, 118, 129, 259
  C. argyrosperma, 124
  C. argyrosperma subsp. *argyrosperma,* 8
  C. lundelliana, 15
  C. okeechobeensis, 99, 100
  C. okeechobeensis subsp. *martinezii,* 20
  C. okeechobeensis subsp. *okeechobeensis,* 16, 118, 129
  C. pepo, 6, 15, 37, 98
  C. pepo subsp. *ovifera,* 8, 98, 124
  C. pepo subsp. *pepo,* 124
Cuphea, 120, 259, 283
Cupressus, 117
Cynara, 259
Cyperus spp., 259
Cypripedium, 157

D
Dactylis, 259
Dahlia, 70, 73, 75, 76
Dalea purpurea, 44, 47

Datura stramonium, 42
Daucus spp., 14, 120, 259
  D. carota, 16, 42, 179
  D. carota L. subsp. *sativus,* 299
Diervilla lonicera, 121
Digitaria, 13, 14, 259
Dioscoreaceae, 75
Dioscorea spp., 259
  D. remotiflora, 76
Diospyros spp., 259
  D. kaki, 124
  D. nigra, 8
Diplotaxis, 13, 14, 259

E
Echeveria, 70, 73, 75, 76
Echinacea spp., 5, 105, 259, 301
  E. angustifolia, 105
  E. purpurea, 8
Echinochloa spp., 13, 14, 259
Elaeis guineensis, 174
Elymus spp., 50, 118, 259, 283, 300
  E. alaskanus, 284
  E. albicans, 284
  E. arizonicus, 284
  E. bakeri, 284
  E. canadensis, 126, 284
  E. curvatus, 284
  E. donianus, 284
  E. elymoides, 284, 297
  E. glabriflorus, 285
  E. glaucus, 285
  E. hirsutus, 285
  E. hystrix, 285
  E. interruptus, 285
  E. lanceolatus, 286
  E. macgregorii, 285
  E. macrourus, 287
  E. multisetus, 286
  E. repens (L.) Gould, 42
  E. riparius, 286
  E. scribneri, 286
  E. sierrae, 287
  E. stebbinsii, 286
  E. trachycaulus, 47, 286
  E. violaceus, 287
  E. virginicus, 287
  E. wawawaiensis, 287
  E. wiegandii, 287
Eragrostis, 259
Ericaceae, 75, 123
Eruca, 13, 14, 259
Eugenia, 259
Euphorbiaceae, 75
Euphorbia spp., 75, 76, 120

F
Fabaceae, 74, 78, 99, 123, 197
Fagopyrum spp.
  F. esculentum, 54
  F. tataricum, 54
Fagus, 259
Festuca spp., 105, 118, 259
  F. pratensis, 299
  F. hallii, 47
Ficus spp., 259
  F. carica, 123
Foeniculum, 260
Fouquieriaceae, 74
Fragaria spp., 13, 14, 102, 120, 127, 259
  F. ×ananassa, 8, 99, 102, 124
  F. cascadensis, 102
  F. chiloensis, 15, 16
  F. chiloensis subsp. *lucida,* 16
  F. chiloensis subsp. *pacifica,* 16
  F. vesca, 102
  F. virginiana, 15, 322
  F. virginiana subsp. *glauca,* 16
  F. virginiana subsp. *platypetala,* 16
  F. virginiana subsp. *virginiana,* 16
Fraxinus, 117, 121

G
Gaylussacia, 259
Gentiana, 157
Glycine spp., 180, 246
  G. falcata, 180
  G. max, 179
Glycyrrhiza, 118, 259
Gossypium spp., 14, 64, 76, 81, 85, 105, 118, 129, 260
  G. arboretum, 81
  G. aridum, 85
  G. gossypioides, 85
  G. harknessii, 15
  G. herbaceum, 81
  G. hirsutum, 6, 8, 81, 85, 124, 179
  G. laxum, 85
  G. lobatum, 85
  G. schwendimanii, 85
  G. trilobum, 85
  G. turneri, 15
Gymnocladus, 130

H

Haliaeetus leucocephalus, 168
Hedysarum, 260
Helianthus spp., 13, 14, 44, 50, 70, 121, 127, 240
  H. angustifolius, 291
  H. annuus, 8, 37, 98–100, 124, 126, 179, 284, 289
  H. anomalus, 20, 240, 289
  H. argophyllus, 289
  H. arizonensis, 20, 290
  H. atrorubens, 291
  H. bolanderi, 289
  H. californicus, 291
  H. carnosus, 129
  H. ciliaris, 292
  H. cusickii, 291
  H. debilis, 16, 289
  H. debilis spp. *vestitus,* 16
  H. decapetalus, 291
  H. deserticola, 16, 289
  H. divaricatus, 15, 16, 291
  H. eggertii, 129, 291
  H. exilis, 20
  H. floridanus, 291
  H. giganteus, 15, 16, 292
  H. glaucophyllus, 292
  H. gracilentus, 292
  H. grosseserratus, 292
  H. hirsutus, 15, 292
  H. laciniatus, 293
  H. laevigatus, 293
  H. longifolius, 293
  H. maximiliani, 15, 16, 293
  H. microcephalus, 293
  H. mollis, 294
  H. neglectus, 290
  H. niveus, 15, 16, 290
  H. niveus subsp. *tephrodes,* 20, 129
  H. nutallii, 51, 294
  H. occidentalis, 294
  H. paradoxus, 129, 138, 290
  H. pauciflorus, 15, 16, 294
  H. petiolaris, 19, 126, 240, 290
  H. petiolaris subsp. *canescens,* 240
  H. praecox, 290
  H. pumilus, 295
  H. resinosus, 295
  H. salicifolius, 295
  H. schweinitzii, 129, 295
  H. silphioides, 295
  H. simulans, 295
  H. smithii, 295
  H. strumosus, 295
  H. tuberosus, 8, 15, 16, 37, 44, 296
Hesperaloe spp., 119
Hevea brasiliensis, 256, 265
Hibiscus, 120, 260
Hordeum spp., 13, 44, 50, 118, 246, 260
  H. pusillum, 6, 98
  H. vulgare, 179
Humulus spp., 260, 274
  H. lupulus, 46
  H. lupulus var. *neomexicanus,* 121
Hydrastis spp., 119, 260
  H. canadensis, 105, 131, 157
Hylocereus, 75, 76
Hymenocallis, 75
Hypericum spp., 260

I

Ilex spp., 14, 260
  I. collina, 129
  I. paraguariensis, 123
Illicium spp., 260
  I. verum, 124
Iochroma, 169, 170
Ipomoea spp., 14, 65, 70, 88, 121, 127, 171, 172, 260, 361
  I. austinii, 172
  I. batatas, 15, 99, 124, 172
  I. batatas var. *batatas,* 124
  I. cordatotriloba, 15, 16, 172
  I. lacunosa, 172
  I. leucantha, 15, 16, 172
  I. littoralis, 16
  I. ramosissima, 15
  I. tenuissima, 16
  I. tiliacea, 15
Iridaceae, 74
Iva anuua var. *macrocarpa,* 8, 98

J

Jaltomata spp., 170, 180, 181
  J. aijana, 180
  J. antillana, 180
  J. incahuasina, 180
  J. sinuosa, 180
Jatropha, 70, 75–77, 260
Juglandaceae, 74, 123, 125
Juglans spp., 13, 14, 104, 119, 260
  J. californica, 132
  J. cinerea, 105, 119
  J. hindsii, 105

  J. major, 119
  J. microcarpa, 119
  J. nigra, 105, 119, 125
  J. regia, 104–105
Juniperus, 117

K
Kalmia, 130
Koeleria macrantha, 52
Krascheninnikovia lanata, 47

L
Lactuca spp., 13, 14, 54, 119, 260
  L. floridana, 126
  L. saligna, 15, 16
  L. sativa, 123, 302
Lamiaceae, 74
Lathyrus, 14, 116, 118, 260
Lauraceae, 74
Lepidium, 50, 260
Lespedeza, 118, 260
Leymus spp., 260, 283, 300
  L. ajanensis, 287
  L. ambiguus, 287
  L. californicus, 287
  L. cinereus, 287
  L. condensatus, 287
  L. flavescens, 288
  L. innovatus, 288
  L. mollis, 288
  L. pacificus, 288
  L. salina, 297
  L. simplex, 288
  L. triticoides, 288
Licania, 260
Lilium, 119, 260
Limnanthes spp., 105, 120, 260, 274
Linum, 260
Lolium spp., 260, 299
  L. perenne, 299
Lomatium, 302, 303
Lonicera caerulea, 39, 47
Lotus, 105, 118, 197, 360
Lupinus, 14, 102, 116, 118, 127, 260
Lythraceae, 74

M
Magnolia spp., 130
  M. ashei, 121
Malpighiaceae, 74
Malus spp., 13, 14, 118, 260, 319
  M. angustifolia, 118
  M. coronaria, 118
  M. fusca, 15, 16, 118
  M. sieversii, 264
Malvaceae, 74, 124
Manihot spp., 14, 70, 76, 88, 260
  M. aesculifolia, 15
  M. angustiloba, 15, 16
  M. esculenta, 179
  M. rubricaulis, 15
  M. walkerae, 15, 16, 20, 129, 138
Manilkara, 260
Martyniaceae, 74
Medicago spp., 14, 44, 260, 301
  M. sativa, 16, 179
  M. sativa subsp. *sativa,* 303
Melilotus, 260, 302
Mentha, 44, 119, 260
Mespilus, 260
Micromonolepsis, 119
Monarda spp., 44
  M. fistulosa, 51
Monardella, 119
Morus, 260
Myrtaceae, 74, 123

N
Nasturtium, 260
Nicotiana spp., 116–117, 129
  N. rustica, 37
  N. tabacum, 175
Nypa fruticans, 174

O
Oenothera, 119
Olea, 260
Onagraceae, 74, 303, 304
Onobrychis, 302
Oplopanax, 260
Opuntia, 8, 64, 120, 260
Orchidaceae, 70, 74, 125
Oryza spp.
  O. glaberrima, 179
  O. sativa, 179
  O. rufipogon, 303
Oxalis spp., 261
Oxytropis, 118

P
Pachyrhizus spp., 8, 261
  P. erosus, 8

Panax spp., 119, 261
  P. ginseng, 131
  P. quinquefolius, 105
Panicum spp.
  P. hirticaule, 8, 98
  P. hirticaule var. *hirticaule,* 98
  P. virgatum, 9, 126
Parthenium spp., 9, 105, 120, 261
  P. argentatum, 9, 105
  P. incanum, 178, 184
Papaver spp., 261
Pascopyrum smithii, 47
Paspalum spp., 118, 261
Passiflora, 261
Pastinaca spp., 42, 261
  P. sativa, 42
Pedaliaceae, 74
Pennisetum, 261
Penstemon spp., 261, 283, 297
  P. eatonii, 297
Persea spp., 6, 9, 14, 64, 70, 76, 82, 88, 121, 261
  P. americana, 6, 9
Phalaris spp., 98, 261
  P. caroliniana, 98
Pharbitis, 171
Phaseolus spp., 6, 9, 14–16, 37, 64, 65, 70, 76–78, 80, 88, 98, 99, 116, 120, 122, 123, 129, 179, 261, 299
  P. acutifolius, 9, 77, 81, 98, 120, 123
  P. acutifolius var. *acutifolius,* 98, 123
  P. albescens, 16
  P. coccineus, 9, 77, 81
  P. dumosus, 81
  P. coccineus var. *griseus,* 9
  P. grayanus, 77
  P. hintonii, 15
  P. longiplacentifer, 15
  P. lunatus, 9, 77, 81
  P. magnilobatus, 77
  P. microcarpus, 77
  P. parvifolius, 77
  P. polystachios, 120, 122
  P. polystachios subsp. *polystachios,* 122
  P. salicifolius, 295
  P. vulgaris, 9, 37, 77, 80, 81, 121, 179, 299
Phleum, 261
Phlox spp., 5, 9, 119, 261
  P. floridana, 118, 126
  P. paniculata, 9
Phoenix dactylifera, 174
Phylloxera vitifoliae, 100
Physalis spp., 9, 65, 76, 86, 129, 261, 301
  P. angulata, 86
  P. philadelphica, 9, 86
Physaria spp., 105, 120, 261, 283
  P. fendleri, 105
Phytolaccaceae, 74
Pinus, 117, 261
Piper, 47, 261, 284
Pistacia spp., 14, 121, 124, 246, 261
  P. vera, 124
Poaceae, 74, 99, 123, 124, 263
Plantaginaceae, 74
Poa spp., 105, 118, 261
Podophyllum, 9
Polygonaceae, 74
Portulaca, 44, 70, 74, 76, 261
Portulacaceae, 74
Pouteria spp., 9, 70, 73, 76, 261
  P. sapota, 9, 73
Prosopis, 120, 261
Prunus spp., 13–16, 20, 99, 104, 120, 124, 126, 127, 129, 138, 146, 261, 319
  P. americana, 15, 16, 126
  P. andersonii, 120
  P. angustifolia, 104, 126
  P. caroliniana, 98
  P. emarginata, 15, 16, 126
  P. fasciculata, 120
  P. geniculata, 129, 138
  P. havardii, 20
  P. ilicifolia, 120
  P. maritima, 15, 16, 20, 121, 126
  P. minutiflora, 15, 16
  P. nigra, 8
  P. pumila, 15, 16
  P. rivularis, 16, 20
  P. subcordata, 120
  P. umbellata, 120
  P. virginiana, 126
Psathyrostachys, 261
Pseudoroegneria spp., 118, 261, 299
  P. spicata, 299
Psidium spp., 9, 70, 76, 121, 123, 261
  P. guajava, 9, 123
Pueraria, 261
Pyrus, 14, 16, 261

R
Raphanus spp., 14, 42, 261
  R. raphanistrum, 42
Rhamnaceae, 74
Rhodiola, 44
Rhododendron, 130, 261

Ribes spp., 13, 14, 47, 104, 120, 123, 129, 261
Ribes spp.
  R. echinellum, 129
  R. hirtellum, 104
  R. nigrum, 104, 123
  R. rubrum, 104, 123
  R. uva-crispa, 104, 123
Rorippa, 13, 14, 261
Rosa, 46, 47, 157, 262
Rosaceae, 76, 124
Rubus spp., 9, 47, 54, 104, 120, 124, 146, 262
  R. allegheniensis, 124
  R. argutus, 104, 124
  R. chamaemorus, 54
  R. idaeus, 47, 104, 124
  R. idaeus subsp. *strigosus,* 104
  R. idaeus var. *strigosus,* 104
  R. occidentalis, 9, 104, 124
  R. pensilvanicus, 124
  R. ursinus, 124
Rudbeckia spp., 5, 119, 262
Ruellia, 262
Rumex, 262

S
Sabal bermudana, 174
Saccaruum spp., 14, 262
Saccharum, 14, 262
Salsola, 262
Salvia hispanica, 9
Sambucus, 119, 120, 262
Saponaria vaccaria, 44
Sapotaceae, 75
Satureja, 262
Schizachyrium spp., 118
  S. sanguineum, 47
  S. scoparium, 47
Scorzonera, 262
Scutellaria, 119
Secale, 44
Sechium spp., 64, 70, 76
  S. edule, 10
Setaria spp., 14, 262
  S. faberi, 15
  S. parviflora, 10
Silene, 180
Silphium integrifolium, x
Simmondsiaceae, 75
Simmondsia spp., 70
Sinapis, 14, 50
Solanaceae, 75, 124, 170
Solanum spp., 14, 64, 76, 81, 120, 169, 170, 262, 283
  S. cardiophyllum, 86
  S. clarum, 15
  S. ehrenbergii, 86
  S. elaeagnifolium, 184
  S. hintonii, 15
  S. hjertingii, 15
  S. hougasii, 15
  S. incanum, 178, 184
  S. insanum, 178, 184
  S. jamesii, 120
  S. lichtensteinii, 178, 184
  S. lidii, 178
  S. linnaeanum, 178, 184
  S. lycopersicum, 81, 175, 182
  S. macrocarpon, 177, 179
  S. melongena, 175, 177–179, 183, 184
  S. pennellii, 182
  S. pimpinellifolium, 81
  S. pyracanthos, 184
  S. tomentosum, 178, 184
  S. torvum, 184
  S. tuberosum, 124, 175, 179
  S. vespertilio, 178
  S. violaceum, 178, 184
Solidago, 157
Sorbus, 262
Sorghastrum spp., 118
Sorghum spp., 14, 112, 120, 179, 246, 262
  S. bicolor, 179
Spondias, 70, 75, 76
Stenocereus, 76
Stictocardia, 170
Stillingia, 262
Stokesia, 119, 120
Streptanthus, 180
Suaeda, 70
Symphyotrichum, 157
Syzygium, 262

T
Tagetes, 65, 76, 129
Taxon, 7–12, 50, 85, 100, 114, 115, 129, 146, 170, 171, 177, 184, 192, 214, 218, 241, 249, 253
Theobroma spp., 6, 14, 65, 70, 76, 262
  T. cacao, 6, 10, 15, 70, 76
Thermopsis, 48
  T. rhombifolia, 48
Thinopyrum spp., 262, 320
  T. intermedium, 320

Thlaspi spp., 262
    T. arvense, 49
Tigridia, 70, 75, 76
Tillandsia, 70, 76
Tragopogon, 262
Triadica, 262
Trifolium, 105, 116, 118, 197, 262, 301
Tripsacum spp., 14, 65, 73, 79, 88, 119, 262
    T. dactyloides, 15, 16, 126
    T. dactyloides var. *dactyloides,* 16
    T. intermedium, 320
    T. latifolium, 320
Triticum spp., 44
    T. aestivum, 169, 179, 283, 284

V
Vaccinium spp., 99, 101, 120, 262
    V. angustifolium, 101, 123
    V. corymbosum, 10, 101, 123
    V. darrowii, 101
    V. elliottii, 101
    V. macrocarpon, 10, 39, 101, 137, 140
    V. myrtilloides, 39
    V. ovalifolium, 125
    V. oxycoccos, 53, 102, 137, 140, 160
    V. scoparium, 47
    V. tenellum, 101
    V. virgatum, 101, 123
Vanilla, 5, 6, 64, 70, 75–77, 125, 262
Vernicia, 262
Viburum trilobum, 47
Vicia, 14, 116, 118, 262
Vigna spp., 262
Vitaceae, 75, 125
Vitis spp., 13, 14, 70, 76, 100, 102, 119, 146, 262, 319, 320
    V. aestivalis, 15, 102, 119
    V. californica, 16
    V. cinerea, 16, 102, 119
    V. cinerea var. *helleri,* 16
    V. labrusca, 16, 102, 119
    V. monticola, 16, 119
    V. mustangensis, 16, 119
    V. palmata, 119
    V. riparia, 47, 100, 102, 119
    V. rotundifolia, 102, 119
    V. rotundifolia var. *rotundifolia,* 102, 119
    V. rupestris, 100, 102, 132
    V. shuttleworthii, 119
    V. vulpina, 119

X
Xanthosoma spp., 14

Z
Zea spp., 14, 64, 65, 129
    Z. diploperennis, 15, 75, 80, 84, 203
    Z. luxurians, 16
    Z. mays, 6, 37, 70, 80, 123, 179, 320
    Z. mays L. subsp. *mays,* 65, 123
    Z. mays subsp. *mexicana,* 80, 283
    Z. mays subsp. *parviglumis,* 80, 84
    Z. perennis, 80, 84
Zizania spp., 54, 127, 146, 246, 262
    Z. aquatica, 37, 54, 103, 300
    Z. palustris, 10, 37, 57, 103, 125, 160
    Z. texana, 100, 103, 126, 127, 129, 248, 275