

Relevance of the genetic structure of natural populations, and sampling and classification approaches for conservation and use of wild crop relatives: potato as an example

Elsa L. Camadro

Abstract: Crop wild relatives (CWRs) are wild taxa with close genetic relationships to species with direct socioeconomic importance. As essential components of natural habitats and agricultural systems, their conservation and sustainable use are vital. CWRs are ex situ conserved in germplasm banks as samples of natural populations (accessions). Most accessions have been assigned specific status according to the Taxonomic Species Concept, which presupposes that living organisms are at the end of speciation. Thus, the morphological and genetic variability that could be encountered in natural populations is disregarded, negatively affecting conservation of allelic frequencies and effective use in breeding. Passport information usually contains collection date and geographical data, but not reproductive behavior of the sampled population (which defines its genetic structure) and sampling strategies. For ex situ multiplications of original collections, no information is provided on the effectively used strategies. As information on how accessions were composed is lacking, conservation of the allelic frequencies from the originally sampled populations is unknown. Knowledge of reproductive biology of plant populations is of utmost importance to understanding their natural morphological and molecular variability and to developing appropriate methodological approaches for sampling, classifying, and ex situ multiplying to conserve gene frequencies for basic and applied purposes.

Key words: crop wild relatives, accessions, population genetic structure, sampling strategies.

Résumé : Les parents sauvages des cultures (PSCs) sont des taxons naturels étroitement reliés, génétiquement, aux espèces d'importance socio-économique directe. Comme composantes essentielles des habitats naturels et des systèmes agricoles, leur conservation et leur utilisation durable sont vitales. On conserve les PSCs ex situ dans des banques de germplasm comme échantillons des populations naturelles (accessions). On assigne à la plupart des accessions un statut spécifique selon le concept des espèces taxonomiques, ce qui présuppose que les organismes vivants constituent le terme de la spéciation. Ainsi, la variabilité morphologique et génétique qu'on pourrait rencontrer dans les populations naturelles n'est pas considérée, ce qui affecte négativement la conservation des fréquences alléliques et leur utilisation efficace en amélioration génétique. L'information des passes ports comporte généralement les données des récoltes et les données géographiques, sans référence au comportement reproductif de la population échantillonnée (définissant sa structure génétique) ni aux stratégies d'échantillonnage. Pour les multiplications ex situ des collections originales, il n'existe aucune information sur les stratégies effectivement utilisées. En absence d'information sur la façon selon laquelle on compose les accessions, on ignore la conservation des fréquences alléliques existantes dans les populations originalement échantillonnées. La connaissance de la biologie reproductive des populations de plantes revêt la plus grande importance pour comprendre leur variabilité morphologique et moléculaire et pour développer des approches méthodologiques d'échantillonnage ainsi que la classification et la multiplication ex situ, afin de conserver les fréquences des gènes pour des fins fondamentales et appliquées.

Mots-clés : parents sauvages des cultures, accessions, structure génétique des populations, stratégies d'échantillonnage.

[Traduit par la Rédaction]

Received 9 May 2012. Accepted 14 August 2012. Published at www.nrcresearchpress.com/cjb on 22 October 2012.

E.L. Camadro. Estación Experimental Agropecuaria Balcarce, Instituto Nacional de Tecnología Agropecuaria (INTA) – Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata (UNMdP); Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ruta Nacional 226 km 73.5, Balcarce, Buenos Aires, Argentina.

E-mail for correspondence: ecamadro@balcarce.inta.gov.ar.

Introduction

A crop wild relative (CWR) is a wild taxon with a close genetic relationship (and, therefore, able to exchange genetic material) with a species of direct socioeconomic importance such as food, fodder, forage, and industrial crops and medicinal, ornamental, and forestry taxa. CWRs are essential components of natural habitats and agricultural systems and important reservoirs of genes of interest for applied purposes. Thus, their conservation and sustainable use is of vital importance for improving agricultural production, increasing food security, and maintaining the environment (<http://www.grfa.org.uk/faq/plants/index.html>).

There is a wide range of crops (e.g., wheat (*Triticum aestivum*), maize (*Zea mays*), rice (*Oryza sativa*), barley (*Hordeum vulgare*), potato (*Solanum tuberosum*), cassava (*Manihot esculenta*), and grain legumes such as *Phaseolus*, *Vicia*, *Vigna*, *Lens*, *Lathyrus*, and *Cicer*) with desirable traits that have been incorporated from CWRs through conventional breeding methods (<http://www.grfa.org.uk/faq/plants/index.html>). Wild potatoes, in particular, have contributed many genes of interest to potato breeding. In fact, many European and North American cultivars have resistance or tolerance to various pathogens and harmful insects introgressed from wild germplasm (Ross 1986; van Berloo et al. 2007; <http://www.europotato.org/>). Furthermore, some wild species also have desirable alleles at genes important for culinary and industrial purposes (Huamán et al. 2000; Santini et al. 2000; Davies et al. 2002; Oltmans and Novy 2002; Jansky and Peloquin 2006). Notwithstanding, the necessity of increasing the use of the CWR genetic pool to minimize crop vulnerability and to cope with environmental changes being brought about by global warming is a major concern of international groups and governments (<http://www.croptrust.org>).

Breeders have access to CWRs through germplasm banks. These banks were created in the 20th century with the objective of conserving samples of the natural variability. Subsequently, and with the need to widen the genetic base of major crops for breeding purposes and to search for metabolites of industrial and (or) pharmaceutical value, germplasm banks became active in the provision of CWRs in the form of “accessions”. Accessions are samples of natural populations ex situ conserved as either original collections or multiplications of original collections, either as botanical seeds of sexually reproducing plants (most frequent) or as vegetative organs (maintained in vivo or in vitro) of asexually reproducing plants or specific genotypes (gene combinations).

Passport information usually contains collection date of the accession, locality, latitude, longitude, and altitude. However, for the oldest accessions, usually only locality and (or) country of origin are reported (see examples in <http://www.grfa.org.uk/search/plants/index.html>, <http://www.ars.usda.gov/Main/docs.htm>, and <http://www.ars-grin.gov/npgs/holdings.html>). Thus, information is unavailable or lacking on the reproductive behavior of the originally sampled population, the number and spatial distribution of sampled plants or seedlings at the collection site, the number of fruits, tubers, or other reproductive organs sampled per plant, the number of sampled plants per population, and how the accession was composed (e.g., for a seed accession, by pooling either similar or different numbers of seeds from either a few or many plants, or by pooling seeds

from either one or various fruits of one or more plants). For ex situ multiplications of seeds, tubers, or other vegetative organs, there is no information on the effective number of parental plants, the number of harvested fruits or vegetative organs per plant, the number of harvested seeds or fruit, and the composition of the accession. Consequently, when working with accessions from germplasm banks, it is not feasible to know whether they are representative samples of the original populations (that is, they conserve their allelic frequencies). Moreover, when working with ex situ multiplications, even if unwanted pollen and seed contamination can be discarded, it cannot be known if genetic drift — that is, a random change in the gene pool of a small population that can lead to the loss of heritable traits — has been avoided. Natural populations, subjected to indigenous selective forces, have been sampled usually only once (Erazzú et al. 2009), so an accession can be considered to be a “snapshot” of what occurred in nature at a given site and moment.

The objective of this commentary, focused on wild potatoes as an example of CWRs but applicable to other taxa, is to demonstrate that (i) the knowledge of the reproductive biology of a plant group and its consequences in the functioning of natural populations is of utmost importance to understanding the morphological and molecular variability encountered in nature and (ii) the methodological approaches used for sampling, classifying, and ex situ multiplying has direct consequences on conservation of gene frequencies of the originally sampled populations (by either increasing or decreasing the likelihood of gene loss or genetic erosion) and the realized breeding progress, which also has an impact on labor efficiency and operational costs.

Plant classification

In the 18th century, the Swedish botanist von Linneus proposed an artificial binomial system for plant classification based on morphological phenotypes (the Taxonomic Species Concept). With minor modifications, this pre-Darwinian system is still in use, with the recent aid of biochemical and molecular tools that are, nevertheless, applied to organisms that had been classified previously according to morphology (see Camadro et al. 2012). In this regard, the two main concepts of species in use are the Taxonomic Species Concept and the Biological Species Concept (Grant 1981), although various other concepts have been developed (Mayr 2000).

The Taxonomic Species Concept entails the use of “holotypes”, which are single physical examples or illustrations of organisms known to have been used when the species or low-ranked taxon were formally described, and is applicable to both uni- and bi-parental organisms. The Biological Species Concept, on the other hand, is based on reproductive relations and morphological discontinuities and was developed for (and applies only to) biparental organisms. Although two biological species are unable to cross and interchange genetic material, two taxonomic species can interbreed and produce fertile progeny. The biological species can coincide or not with the taxonomic species, e.g., “sister species” of *Gilia* are morphological indistinguishable (one taxonomic species) but are unable to cross (two biological species). Thus, if the two uses of the term “species” are not distinguished, the confusion is perpetuated (Grant 1981).

Wild potatoes

Geographical and ecological distribution

Wild potatoes are endemic to the Americas, growing from southwestern USA, through Mexico and Central America, to South America. They occur along the Andes and on Chiloé Island in Chile and towards the east in Argentina, Brazil, Paraguay, and Uruguay (Hawkes 1990; Hijmans and Spooner 2001), from coastal deserts and humid subtropical lowlands to highlands, in a wide variety of micro- and macro-environments.

Taxonomy and field collections

Potatoes (*Solanum* sp.) belong to the *Solanaceae* family, which contains a very large number of herbaceous and arbusive species (Hawkes 1990). Germplasm collection started in the mid-18th century, linked to pioneer botanical explorations carried out by European botanists and explorers, most of whom — and as part of their general botanical collections — made only one or a few gatherings. From the late 19th century to the early decades of the 20th century, increasing efforts were made to collect whole floras for assessment of botanical resources, accompanied by detailed surveys of the natural vegetation. Overlapping with this period, specialized collecting expeditions were made with the primary or only objective of gathering wild potatoes. Principally, the Russian botanist and plant breeder Nikolai Vavilov focused his interest on collecting tubers and seeds of genetic materials with potential breeding value. For collections made mostly in the 18th century, exact localities and dates are frequently lacking; therefore, the historical aspects of the collections had to be reconstructed from herbarium labels and, partly, from biogeographical information (Hawkes and Hjerting 1969).

In the 19th century, various systems were proposed to systematically organize the genus *Solanum* into sections (in Hawkes 1944) or subgenera (in Magoon et al. 1962). One of these sections, *Tuberarium*, was subdivided into the subsections *Basarthrum* and *Hyperbasarthrum* (actually *Potatoe* G. Don) by the German botanist G. Bitter, who used two morphological characters as the criteria (position of pedicel articulation and hair structure). Working exclusively with dry herbarium materials and one or a few specimens per collection, he thought of *Solanum* species as very narrow morphological units with little morphological variation and assigned specific, subspecific, or varietal rank to even slight morphological variants. The Russian botanists and plant breeders Juzepczuk and Bukasov followed Bitter's methods fairly closely but based their descriptions on living materials that were grown in Leningrad (in Hawkes and Hjerting 1969).

By the mid-20th century and due to the great phenotypic variability encountered in potato collections, Hawkes (1963) found it necessary to profoundly revise their classification to avoid a situation in which almost any new collection would be classified as a new species. In relation to classification in this group of plants, Hawkes and Hjerting (1969) wrote "... even from herbarium studies it is possible to see that species had to be regarded as larger units, comprising a wide range of infraspecific variability which could only rarely be fitted into the conventional pattern of varieties and forms ... potato species ... are highly variable genotypically and of extreme phenotypic plasticity" and concluded that "... Environmental differences are sufficient to cause great phenotypic differences, sometime overriding or obscuring the genotypic

ones ...". These conclusions had been independently reached by Correll (1962).

In the late 20th and early 21st centuries, various taxonomic revisions were carried out that were based, for the most part, on herbarium specimens and samples of living plants and the application of molecular tools to accessions that had been previously assigned the category of species based on morphological phenotypes. Thus, in the past 20 years, the number of tuber-bearing species was reduced from 227 (Hawkes 1990) to 203 (Hijmans and Spooner 2001) to 189 (Spooner and Salas 2006), finally "converging around" 110 (Spooner 2009).

Ploidy and genome differentiation

Wild and cultivated potatoes form polyploid series with $2n = 2x, 3x, 4x, 5x, 6x$ ($x = 12$) chromosomes, with most species being diploid. There is scarce structural genome differentiation in this group of plants, as revealed by the results of chromosome pairing and fertility studies. These results led Matsubayashi (1991) to postulate the presence of an A genome from which four genomes (B, C, D, and E) were derived in the course of evolution. Thus, spontaneous fertile hybrids are frequently formed in nature (Masuelli et al. 2009). Moreover, it is feasible to incorporate small chromosome segments carrying desirable agronomic traits into the cultivated gene pool, increasing allelic diversity, with the consequent increase in the number of intra- and inter-locus interactions, which are positively correlated with yield (Mendiburu et al. 1974; Jansky and Peloquin 2006; Carputo et al. 2006). In this regard, Camadro et al. (2004) discussed the evidence supporting the assertion that pollen–pistil incompatibility and endosperm abortion in crosses, plus male sterility in the resulting progenies when prezygotic or endosperm barriers are either absent or incomplete, have been substitutes of genome differentiation in the evolution of wild potatoes, and Camadro et al. (2012) called attention to the necessity of revising the species concept in this group of plants.

Reproductive behavior

Although the preponderant mode of reproduction in nature has not been ascertained, wild potatoes can reproduce both sexually (by seeds) and asexually (by stolons and tubers). Because they possess a one multiallelic S locus conferring gametophytic self-incompatibility, which prevents fertilization if there is identity between S alleles carried by pollen and pistil, diploids are obligate outcrossers (allogamous). On the other hand, polyploids can be either allogamous, with a variable percentage of autogamy (self-fertilization), or autogamous. In this regard, inhibition of the self-incompatibility reaction in polyploids can occur in pollen grains that are heterozygous at the S locus, a phenomenon that Frankel and Galun (1977) referred to as competitive interaction.

Pollination in allogamous wild diploid and polyploid potatoes is entomophilous (insect pollinated). The main flower visitors are species of *Bombus* (bumbees), which can typically forage over 100–1750 m (Walther-Hellwig and Frankl 2000), facilitating gene flow among sympatric populations. Seeds can be dispersed by water, anthropic activities such as soil movements (principally during road construction and maintenance), and animals (mammals and birds) that can carry them in their digestive tracts after eating the palatable fruits, even over long distances. In northwestern Argentina, it is very

common to observe phenotypically very variable potato populations growing in animal enclosures.

Heritable meiotic abnormalities

Genetically controlled abnormalities in meiosis result in the production of $2n$ gametes ($2n$ pollen and (or) $2n$ eggs), which are gametophytes or gametes with sporophytic chromosome numbers (Mok and Peloquin 1975; Mendiburu et al. 1974). These gametes are functional in intra- and inter-ploid crosses, a phenomenon known as sexual polyploidization. Moreover, it is likely that haploids (sporophytes with gametophytic chromosome numbers), which can be regularly obtained by controlled interploid crosses (Peloquin and Hougas 1959), are formed spontaneously in nature. In this way, sexual polyploidization and depolyploidization provide for spontaneous gene flow and introgression among ploidy levels (see Camadro et al. 2004).

Hybridization barriers

In nature, wild potatoes are isolated by external (spatial, ecological, and geographical) hybridization barriers (Hawkes and Hjerting 1969), but also by internal barriers, which reside in the plant tissues themselves (Camadro et al. 2004). The internal barriers can be either prezygotic (cross-incompatibility) or postzygotic (abortion of embryo, endosperm or both, inviability, weakness or sterility of the F_1 hybrid and (or) the F_2 and advanced segregating generations). Hybridization barriers in the group are under genetic control, but the molecular bases of the pre- and post-zygotic endosperm barriers have not been elucidated. Notwithstanding, it has been postulated that there are alternative forms (alleles) of the genes involved in expressing these barriers that segregate in meiosis whereby some genotypic combinations may be compatible, allowing hybridization and introgression (Camadro and Peloquin 1981; Ehlenfeldt and Hanneman 1988; Camadro and Masuelli 1995; Johnston and Hanneman 1996).

The breeding system of wild potatoes provides, then, for the maintenance of superior genotypes in stable environments and the evolution of new genotypic combinations, as well as the overlapping of generations (parental, hybrid, backcross) similar to what has been observed in *Iris* (Taylor et al. 2009). Morphological phenotypic differences among taxa are not of great magnitude, and hybridization and gene flow within and between ploidy levels and generations produce very complex patterns of morphological variation (Hawkes 1963; Correll 1962; Ugent 1966; Masuelli et al. 2009). The implications of the reproductive biology of the group in germplasm utilization has been discussed, among other authors, by Hanneman (1999), Carputo et al. (2006), and Ortiz (2010).

Genetic structure of spontaneous populations, sampling strategies, and classification

Accessions of wild potatoes are usually assigned taxonomic status without taking into consideration that the natural populations from which they came can consist of either uniparental plants (that is, plants that originated by asexual reproduction = clones) or both uniparental plants (clones) and biparental plants (generated by the sexual process), and that generations (parents, F_1 , backcrosses, and F_2 and more advanced segregating generations) can overlap at a given site and time due to the two modes of reproduction available to them. In angiosperms, in general, natural populations of biparental

plants can exhibit morphological and genetic variations regardless of their preponderant type of sexual reproduction (either allogamy or autogamy). In sexually reproducing populations, each zygote is generated by the fusion of two gametes (one from the male parent and the other from the female parent); thus, there is opportunity for the occurrence of two rounds of genetic recombination: the first in meiosis (through chromosome segregation and gene recombination by crossing-over) and the second at fertilization. Allogamous (cross-pollinating) plants are highly heterozygous; thus, each plant in a population is a particular genotype. Autogamous (self-pollinating) plants are highly homozygous; however, a population of an autogamous species can be composed of plants homozygous for either the same or different gene combinations. This is so because allogamy and autogamy are not necessarily strict. Under certain environmental conditions, autogamous plants can cross-pollinate, and if the population is composed of plants homozygous for different gene combinations, heterozygous F_1 plants can be generated. Upon self-pollination, these occasionally generated F_1 plants will produce segregating progenies; after several self-pollination reproductive cycles, the population could be composed of a mixture of plants homozygous for the same (or very similar) gene combinations and homozygous for different gene combinations. Therefore, at a given site and sampling time, a population of an autogamous species may exhibit morphological and genetic variability, as in populations of allogamous species, and even clonal populations when they are composed of clones derived from more than one parental genotype. Moreover, because plant phenotype is the result of genotype \times environment interactions, one given genotype may exhibit different phenotypes when grown under different environmental conditions. This fact was pointed out by Hawkes and Hjerting (1969) when discussing the limitations of both the use of herbarium materials for potato classification and the validity of comparisons of morphological phenotypes when different plants (genotypes) are grown under different environments.

In summary, morphological and genetic variation can be present within and between natural populations regardless of their reproductive behavior, and there could be no correspondence between the observed phenotypic variation and the actual genetic variation of a population. However, under the Taxonomic Species Concept, plants of a given population are classified according to their resemblance to holotypes, not taking into account that homogeneity in morphological phenotypes is expected only in populations of (i) asexually reproducing plants derived from one genotype (clone) and (ii) sexually reproducing self-pollinating plants that are highly homozygous for one genotype when they are growing under similar environmental conditions. Even genetically identical plants growing at the same location (macroenvironment) but under different ecological conditions (microenvironments) may exhibit morphological variability.

Genetic diversity in higher plants

The genetic composition of a population is described by its array of gene frequencies. Gene frequency (also known as allele frequency) is the proportion of one particular type of allele to the total of all alleles at a genetic locus in a breeding population or the probability of finding a specific gene when a gene is randomly chosen from the population (Rieger et al.

1991). As a contribution to the understanding of gene diversity in higher plants, Schoen and Brown (1991) analyzed published gene frequencies at isozyme loci in six self-fertilizing and nine cross-fertilizing species of monocots and dicots belonging to six and four different genera, respectively. Inbreeding species exhibited markedly greater population-to-population variation in Nei's gene diversity statistic (Nei 1973), and estimates of the effective population numbers (N_e) for inbreeding species were more variable than those for outbreeding species. These authors considered that inbreeding species might be composed of a subset of marginal ("sink") populations of limited genetic diversity and evolutionary potential that were derived from a number of ancient and genetically diverse "source" populations of relatively large N_e . These results are similar to the ones reported by other authors in various plant groups, using allozymes, molecular markers, and DNA sequences, and discussed by Charlesworth (2003) on theoretical grounds. It is necessary, however, to point out that many of these studies were carried out with accessions from germplasm banks, which could have the limitations discussed in this commentary. Thus, the development of sampling strategies should be preceded by population diversity surveys to decide how populations will be represented in germplasm collections (Jain (1975) in Schoen and Brown 1991). These surveys may have diminished importance in outbreeding species in which there is comparatively decreased gene diversity variation among populations, as has been reported to occur in wild potatoes by Bedonni and Camadro (2009) and Erazzú et al. (2009).

What to conserve?

The scarce passport information of most CWR accessions in germplasm banks is a reflection of the original purpose of their creation, which was to conserve samples of the natural variability. However, if natural genetic variability is to be effectively conserved and used for applied purposes, gene frequencies of natural populations ought to be taken into account. As a matter of fact, genetic diversity is a function of the number and frequency of alleles per gene in a given gene pool, and this parameter is used to reflect the integration of diversity at individual loci into multilocus genotypes, a process mediated primarily by the mode of reproduction (Rieger et al. 1991). The number of possible genotypes in a population greatly exceeds the number of genes. In a sexually reproducing population in which cross-pollination can occur, the number of genotypes becomes enormous in a short time because genes are reassorted in each generation in meiosis and at fertilization as previously discussed; therefore, the effects of such reassortments are largely transitory and lost as quickly as they are created. Therefore, it is often convenient to consider a natural population not so much as an aggregate of living individuals, but as an aggregate of gene ratios (Crow and Kimura 1970). Thus, changes in the frequency of genes themselves need to be considered for long-range trends because only genes (not genotypes) have continuity from generation to generation (Falconer 1989). In this regard, the genetic structure of a population has to be known if representative samples are to be drawn for both conservation and applied purposes (including crop breeding).

Conclusion

Research in the field of speciation has indicated that there are many different biological and historical conditions that may or may not ultimately lead to lineage divergence or reticulation (Grant 1981; Will and Rudinoff 2004). This is especially true for closely related plant groups in which taxa are in the process of diverging (see Camadro et al. 2012) or have recently diverged and are frequently represented by incomplete genomic rearrangement (Avice 2000). As Will and Rudinoff (2004) stated when discussing the use of DNA barcoding methods in classification versus other types of character data, only holistic views of organisms incorporating phylogeny, functional morphology, reproductive behavior, and ecology, among others, will help to make informed conservation decisions. It has to be taken into account that classification systems and sampling strategies of natural populations are of fundamental importance for the preservation of the natural genetic diversity and its effective use for applied purposes.

Researchers from different disciplinary fields (botany, taxonomy, genetics, evolution, and breeding) need to engage in a discussion — unbiased and thorough — of strategies for classification of CWR populations and sampling to conserve gene frequencies of spontaneous populations *ex situ* and (or) *in situ* with the aim of preventing or avoiding genetic erosion and providing heritable variability for applied purposes.

Proposal

It is my opinion that there are two key issues in devising CWR collection strategies for *ex situ* conservation of gene frequencies of natural populations: breeding relations and environment.

As previously stated, spontaneous populations can be composed of either morphologically homogeneous or heterogeneous plants that could resemble holotypes or have either intermediate or not previously described morphological phenotypes. Holotypes have been described on the basis of qualitative and quantitative morphological characters. Qualitative characters such as shape and color of flowers, leaves, and fruits have relatively simple genetic control, with little or no environmental influence on gene expression, as do measurable characters such as number of petals, sepals, or anthers that may have become fixed during evolution. Other characters (quantitative) are controlled by a few or several genes (polygenes) that individually exert a slight effect on the phenotype, but whose expression is subject to considerable modification by environmental influences (e.g., plant height, leaf size, tuber weight). However, the presence in a population of genes controlling other functions, some of which could be of potential applied value (e.g., content of secondary metabolites, resistance or tolerance to adverse factors), is usually not revealed by plant morphology. Moreover, as has been discussed by Camadro et al. (2012), there are difficulties in trying to relate molecular data, obtained by using the current approaches, to relevant morphological and (or) ecophysiological traits.

Samples of spontaneous populations are incorporated as accessions in germplasm banks, usually with category of species (using the Taxonomic Species Concept). Let us assume that accessions conserve the gene frequencies of the natural

populations from which they come because the appropriate sampling strategies have been applied. Usually, in the process of ex situ maintenance and (or) multiplication of accessions, “off-type” plants are discarded. By doing so, gene frequencies are modified, with the risk of losing potentially valuable allelic variants. However, if plants of a natural population, regardless of their morphological phenotypes, can interbreed, plant morphology is no longer a concern in sampling, maintenance, and conservation of CWR, because genes, not genotypes, are transmitted from one generation to the next, as already discussed, and are the ones that should be preserved.

Accessions of sympatric populations that have been classified as different “species” according to plant morphology can, nevertheless, share genes as the result of gene flow and introgression when at least a few plants are compatible and cross-pollination occurs. Allopatric populations of the same “species”, on the other hand, can vary in their gene frequencies if they are adapted to different environmental conditions. Thus, in my opinion, in applied studies, it is a common mistake to conclude on (or extrapolate results from) one species. In doing so, it is not considered that accessions have been classified on the basis of plant morphology, but that they have come from natural populations that grow in different geographical areas and, frequently, under different environments (e.g., *S. chacoense*, a self-incompatible diploid wild potato, grows in contrasting macro- and micro-environments in Argentina, along a transect approximately 1500 km long, and overlaps with populations of other allogamous wild potato “species” that are partially or completely compatible with it). Consequently, accessions of a given taxonomic species can vary in gene frequencies for adaptation and potentially valuable traits for applied purposes.

Practical issues

Currently, and for the most important crops, the number of CWR accessions in germplasm banks over the world is high, but there is scarce passport information for many of them and “gaps” in geographical areas and environments that have been explored and sampled.

In the following sections, I give my opinion on how to proceed with already existing accessions of wild potatoes (most of them allogamous or predominantly allogamous) and how to make new collections.

Accessions in germplasm banks

Accessions in germplasm banks are the results of the efforts of naturalists and scholars who participated in collection expeditions, carried out over many years with variable purposes. For many accessions (mostly the oldest ones), there is scarce or no passport information on natural population size, sampling strategies, and the environment. In my opinion, and when possible, it would be valuable for curators to seek information on at least macro- and micro-environments, because this information is of great help for applied studies.

Regarding the maintenance and (or) multiplication processes, in addition to the good practices that will help to prevent or reduce unwanted seed and (or) pollen contamination, I consider the following points to be important.

- a. “Off-types” ought to be kept because they are part of the population genetic pool.
- b. Around 15–25 plants have to be used in controlled crosses. At flowering, pollen viability has to be checked, and

pollen–pistil relations between the individual plants have to be determined. This information, in addition to the pollen viability data, will allow a general estimation of the effective parental number, which should be provided to the user.

- c. Similar numbers of seeds from each female parent have to be pooled to compose the population (e.g., first multiplication of the original accession). The number of female parents should be registered, and the user should be informed of this number and of any deviation from the expected equal seed contribution of each of them.

New collections

For new collections, I suggest that collectors should consider the following practices.

- a. Sample only populations composed of at least 15–25 plants, taking into consideration the issues raised in the current commentary on mode of reproduction (sexual and asexual), type of sexual reproduction (allogamy and autogamy), among others.
- b. For composing the accession, choose strategies that would allow the preservation of the gene frequencies of the sampled population. In the field, estimate pollen viability with an easy staining technique, using a portable light microscope. Keep records to provide to the user.
- c. For populations composed of plants with different morphological phenotypes, keep samples (seeds and (or) reproductive organs) of each phenotype separately, but compose the accession with of all plants because they may be part of the same gene pool.
- d. Describe macro- and micro-environments and accompanying flora.
- e. Register any additional data that could be of interest for the user, e.g., presence of a disease or pest in the population but not on some of plants; if found, keep samples of the apparently “tolerant” or “resistant” plants separate, but compose the accession as in (c).
- f. To be able to easily interchange information with other researchers, use quotation marks for the taxonomic name under which the accession is classified (e.g., “*S. chacoense*”).
- g. If possible, make new collections at the same sampled site at intervals of 3–5 years, because the presence of a population at a given site (or close to it) and its genetic composition can be highly dependent on the environmental conditions and the time of the year at which the collection was carried out.
- h. In planning collection strategies, keep in mind that the Taxonomic Species Concept is a human construction that could be poorly reflecting what is actually occurring in nature.
- i. Depending on the availability of money and time, sites of origin of already existing accessions for which passport information is scarce or lacking, or that have presented reproductive problems in the ex situ multiplication process, should be revisited. If populations are still present, they should be sampled using the most appropriate strategies; afterwards, other types of studies should be carried out (see Erazzú et al. 2009) to establish if there is correspondence between the ex situ conserved genetic material and the natural population. This information would allow the development of strategies to either prevent or minimize gene erosion or loss.

Acknowledgements

The author thanks INTA, UNMdP, and CONICET for financing, over the years, the research activities that give support to the ideas and conclusions presented in this paper.

References

- Avise, J.C. 2000. Phylogeography: the history and formation of species. Harvard University Press, Cambridge, Mass.
- Bedonni, M.C., and Camadro, E.L. 2009. Morphological and molecular evidence of natural interspecific hybridization in the diploid potato *Solanum kurtzianum* from Argentina. *Botany*, **87**(1): 78–87. doi:10.1139/B08-116.
- Camadro, E.L., and Masuelli, R.W. 1995. A genetic model for the endosperm balance number (EBN) in wild potato *Solanum acaule* Bitt. and two related diploid species. *Sex. Plant Reprod.* **8**(5): 283–288. doi:10.1007/BF00229385.
- Camadro, E.L., and Peloquin, S.J. 1981. Cross-incompatibility between two sympatric polyploid *Solanum* species. *Theor. Appl. Genet.* **60**(2): 65–70. doi:10.1007/BF00282417.
- Camadro, E.L., Carputo, D., and Peloquin, S.J. 2004. Substitutes for genome differentiation in tuber-bearing *Solanum*: interspecific pollen–pistil incompatibility, nuclear–cytoplasmic male sterility, and endosperm. *Theor. Appl. Genet.* **109**(7): 1369–1376. doi:10.1007/s00122-004-1753-2.
- Camadro, E.L., Erazzú, L.E., Maune, J.F., and Bedogni, M.C. 2012. A genetic approach to the species problem in wild potatoes. [Online.] *Plant Biol. (Stuttg.)*, **14**(4): 543–554. doi:10.1111/j.1438-8677.2012.00563.x. PMID:22372767.
- Carputo, D., Camadro, E.L., and Peloquin, S.J. 2006. Terminology for polyploids based on their cytogenetic behavior: consequences in genetics and breeding. *In Plant Breeding Reviews*. Vol. 26. Edited by J. Janick. John Wiley and Sons, London. pp. 105–124.
- Charlesworth, D. 2003. Effects of inbreeding on the genetic diversity of populations. *Philos. Trans. R. Soc. Lond. B*, **358**(1434): 1051–1070. doi:10.1098/rstb.2003.1296.
- Correll, D.S. 1962. The potato and its wild relatives. Texas Research Foundation, Renner, Tex.
- Crow, J.F., and Kimura, M. 1970. An introduction to population genetics theory. Burgess Publishing Co., Minneapolis, Minnesota.
- Davies, C.S., Ottman, M.J., and Peloquin, S.J. 2002. Can germplasm resources be used to increase the ascorbic acid content of stored potatoes? *Am. J. Potato Res.* **79**(4): 295–299. doi:10.1007/BF02986362.
- Ehlenfeldt, M.K., and Hanneman, R.E. 1988. Genetic control of endosperm balance number (EBN): three additive loci in a threshold-like system. *Theor. Appl. Genet.* **75**(6): 825–832.
- Erazzú, L.E., Camadro, E.L., and Clausen, A.M. 2009. Persistence over time, overlapping distribution and molecular indications of interspecific hybridization in wild potato populations of Northwest Argentina. *Euphytica*, **168**(2): 249–262.
- Falconer, D.S. 1989. Introduction to quantitative genetics. 3rd ed. Longmans Green / John Wiley & Sons, Harlow, Essex, UK, and New York.
- Frankel, R., and Galun, E. 1977. Pollination mechanisms, reproduction and plant breeding. Springer-Verlag, Berlin, Heidelberg, New York.
- Grant, V. 1981. Plant speciation. 2nd ed. Columbia University Press, New York.
- Hanneman, R.E., Jr. 1999. The reproductive biology of the potato and its implication in breeding. *Potato Res.* **42**(2): 283–312. doi:10.1007/BF02357859.
- Hawkes, J.G. 1944. Potato collecting expeditions in Mexico and South America. II. Systematic classification of the collections. *Bulletin of the Imperial Bureau of Plant Breeding and Genetics*, Cambridge, Mass.
- Hawkes, J.G. 1963. A revision of the tuber-bearing *Solanums*. 2nd ed. Scottish Plant Breeding Station Records, Pentlandsfield, UK.
- Hawkes, J.G. 1990. The potato: evolution, biodiversity and genetic resources. Belhaven Press, London.
- Hawkes, J.G., and Hjerting, J.P. 1969. The potatoes of Argentina, Brazil, Paraguay and Uruguay. A biosystematic study. Oxford University Press, Oxford, UK.
- Hijmans, R.J., and Spooner, D.M. 2001. Geographic distribution of wild potato species. *Am. J. Bot.* **88**(11): 2101–2112. doi:10.2307/3558435.
- Huamán, Z., Hoekstra, R., and Bamberg, J.B. 2000. The intergenebank potato database and the dimensions of available wild potato germplasm. *Am. J. Potato Res.* **77**(6): 353–362. doi:10.1007/BF02882289.
- Jansky, S.H., and Peloquin, S.J. 2006. Advantages of wild diploid *Solanum* species over cultivated diploid relatives in potato breeding programs. *Genet. Resour. Crop Evol.* **53**(4): 669–674. doi:10.1007/s10722-004-2949-7.
- Johnston, S.A., and Hanneman, R.E., Jr. 1996. Genetic control of endosperm balance number (EBN) in the *Solanaceae* based on trisomic and mutation analysis. *Genome*, **39**(2): 314–321. doi:10.1139/g96-042.
- Magoon, M.L., Ramanujam, S., and Cooper, D.C. 1962. Cytogenetical studies in relation to the origin and differentiation of species in the genus *Solanum*. *Caryologia*, **15**(1): 155–252.
- Masuelli, R.W., Camadro, E.L., Erazzú, L.E., Bedogni, M.C., and Marfil, C.F. 2009. Homoploid hybridization in the origin and evolution of wild diploid potato species. *Plant Syst. Evol.* **277**(3–4): 143–151. doi:10.1007/s00606-008-0116-x.
- Matsubayashi, M. 1991. Phylogenetic relationships in the potato and its related species. *In Chromosome engineering in plants: genetics, breeding, evolution*. Part B. Edited by T. Tsuchiya and P.K. Gupta. Elsevier, Amsterdam. pp. 93–118.
- Mayr, E. 2000. What makes biology unique? Considerations on the autonomy of a scientific discipline. The Press Syndicate of the University of Cambridge, Cambridge, Mass.
- Mendiburu, A.O., Peloquin, S.J., and Mok, D.W.S. 1974. Potato breeding with haploids and $2n$ gametes. *In Proceedings of the International Symposium on Haploids in Higher Plants*. University of Guelph, Guelph, Ontario, Canada. pp. 249–258.
- Mok, D.W.S., and Peloquin, S.J. 1975. Three mechanisms of $2n$ pollen formation in diploid potatoes. *Can. J. Genet. Cytol.* **17**: 217–225.
- Nei, M. 1973. Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. U.S.A.* **70**(12): 3321–3323. doi:10.1073/pnas.70.12.3321.
- Oltmans, S.M., and Novy, R.G. 2002. Identification of potato (*Solanum tuberosum* L.) haploid \times wild species hybrids with the capacity to cold-chip. *Am. J. Potato Res.* **79**(4): 263–268. doi:10.1007/BF02986359.
- Ortiz, R. 2010. Potato breeding via ploidy manipulations. *In Plant breeding reviews*. Vol. 16. Edited by J. Janick. John Wiley & Sons, Inc., Oxford, UK.
- Peloquin, S.J., and Hougas, R.W. 1959. Decapitation and genetic markers as related to haploidy in *Solanum tuberosum*. *Eur. Potato J.* **2**(3): 176–183. doi:10.1007/BF02365563.

- Rieger, R., Michaelis, A., and Green, M.M. 1991. A glossary of genetics, classical and molecular. 5th ed. Springer-Verlag, Berlin, Heidelberg, New York. [In English.]
- Ross, H. 1986. Potato breeding — problems and perspectives. Verlag Paul Parey, Berlin, Hamburg.
- Santini, M., Camadro, E.L., Marcellán, O.N., and Erazzú, L.E. 2000. Agronomic characterization of diploid hybrid families derived from crosses between haploids of the common potato and three wild Argentinian tuber-bearing species. *Am. J. Potato Res.* **77**(4): 211–218. doi:10.1007/BF02855788.
- Schoen, D.J., and Brown, A.H.D. 1991. Intraspecific variation in population gene diversity and effective population size correlates with the mating system in plants. *Proc. Natl. Acad. Sci. U.S.A.* **88**(10): 4494–4497. doi:10.1073/pnas.88.10.4494.
- Spooner, D.M. 2009. DNA barcoding will frequently fail in complicated groups: an example in wild potatoes. *Am. J. Bot.* **96**(6): 1177–1189. doi:10.3732/ajb.0800246.
- Spooner, D.M., and Salas, A. 2006. Structure, biosystematics, and genetic resources. *In Handbook of potato production, improvement, and post-harvest management. Edited by G.J. Khuranam and S.M. Paul.* Haworth Press, New York. pp. 1–39
- Taylor, S.J., Arnold, M.L., and Martin, N.H. 2009. The genetic architecture of reproduction isolation in Louisiana irises: hybrid fitness in nature. *Evolution*, **63**(10): 2581–2594. doi:10.1111/j.1558-5646.2009.00742.x.
- Ugent, D. 1966. Hybrid weed complexes in *Solanum*, section *Tuberarium*. Ph.D. thesis, The University of Wisconsin, Madison, Wisc.
- van Berloo, R., Hutten, R.C.B., van Eck, H.J., and Visser, R.G.F. 2007. An online potato pedigree database resource. *Potato Res.* **50**(1): 45–57. Available at <http://www.plantbreeding.wur.nl/potatopedigree/> [accessed 9 May 2012]. doi:10.1007/s11540-007-9028-3.
- Walther-Hellwig, K., and Frankl, R. 2000. Foraging distances of *Bombus muscorum*, *Bombus lapidarius*, and *Bombus terrestris* (Hymenoptera, Apidae). *J. Insect Behav.* **13**(2): 239–246. doi:10.1023/A:1007740315207.
- Will, K.W., and Rubinoff, D. 2004. Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. *Cladistics*, **20**(1): 47–55. doi:10.1111/j.1096-0031.2003.00008.x.