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Ex situ PLANT GERMPLASM CONSERVATION REVISED AT THE LIGHT OF MECHANISMS AND METHODS OF GENETICS

CONSERVACIÓN DE GERMPLASMA *ex situ* REVISADA A LA LUZ DE MECANISMOS Y MÉTODOS DE GENÉTICA

Camadro E.L.[1], **Rimieri P.**[2]

[1] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata (FCA, UNMdP), Ruta Nacional 226 km 73.5, 7620 Balcarce, Argentina.

[2] Estación Experimental Agropecuaria Pergamino, Instituto Nacional de Tecnología Agropecuaria (INTA), Avenida Frondizi (Ruta 32) km 4.5, 2700 Pergamino, Argentina.

Corresponding author:
Elsa L. Camadro
camadro.elsa@inta.gob.ar

ORCID 0000-0002-1739-5059

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ABSTRACT

Plant genetic resources for food and agriculture are *ex situ* conserved in germplasm banks as samples (accessions) of natural or naturalized populations, either as the originally sampled propagules (mainly seeds) or their multiplications. The premises underlying *ex situ* conservation are that (a) it is the safest and cheapest alternative for germplasm preservation for future generations and (b) accessions are representative of the genetic diversity encountered in nature. In the past decades, ideas, alternatives and considerations have been put forward on the topic, and protocols have been devised for plant germplasm sampling, conservation and multiplication. However, limitations in the management efficiency of germplasm banks have been pointed out by international organizations. In our opinion, germplasm banks in general need to revise their functioning and management at the light of principles and methods of Genetics. To that end, it is necessary to consider the reproductive biology of higher plants -whose genetic consequences at both the individual plant and the population levels are not always either fully understood or taken into account in devising the protocols-, the genetic structures of wild and cultivated populations, and the course of the genetic material in the populations. In this paper, we discuss the three topics and provide an example of a national forage breeding program, from germplasm bank accessions as the germplasm of origin to the obtainment of commercial cultivars. Finally, we present a proposal as a base for discussion among curators, researchers and breeders.

Key words: accessions, breeding, genetic resources, germplasm banks, population genetics

RESUMEN

Los recursos genéticos vegetales para la alimentación y la agricultura se conservan *ex situ* en bancos de germoplasma como muestras (introducciones) de poblaciones naturales o naturalizadas ya sea como propágulos originales (mayoritariamente semillas) o sus multiplicaciones. Las premisas subyacentes son que (a) es la alternativa más segura y barata de preservación de germoplasma para futuras generaciones y (b) las introducciones son representativas de la diversidad genética que se encuentra en la naturaleza En las últimas décadas, se han presentado ideas, alternativas y consideraciones sobre el tema y se han elaborado protocolos para el muestreo, conservación y multiplicación de germoplasma. Sin embargo, organizaciones internacionales han señalado limitaciones en la eficiencia del manejo de los bancos de germoplasma. En nuestra opinión, se necesita revisar el funcionamiento y manejo de dichos bancos en general a la luz de los principios y métodos de Genética. Para tal fin, es necesario considerar la biología reproductiva de las plantas superiores -cuyas consecuencias genéticas a nivel de planta individual y de población no se comprenden en su totalidad o no se consideran al idear los protocolos -, las estructuras genéticas de poblaciones naturales y cultivadas, y el curso del material genético en las poblaciones. En este trabajo discutimos los tres temas y proveemos un ejemplo de un programa nacional de mejoramiento de forrajeras, desde las introducciones como germoplasma de origen hasta la obtención de cultivares comerciales. Finalmente, presentamos una propuesta como base de discusión entre curadores, investigadores y mejoradores.

Palabras clave: introducciones, mejoramiento genético, recursos genéticos, bancos de germoplasma, genética de poblaciones

INTRODUCTION

With the aim of contributing to the development of coherent and effective strategies for conservation of plant genetic resources for food and agriculture, ideas, alternatives and considerations have been put forward over the years in many methodological publications. Limitations in the management efficiency of germplasm banks, not infrequently carried out without appropriate planning, were pointed out in "The State of the World´s Plant Genetic Resources for Food and Agriculture" (FAO, 1996). In that report, it was considered that over 65% of the worldwide *ex situ* conserved collections needed regeneration. Almost 10 years later, the logistics of germplasm banks was integrally analyzed in the last manual published by Biodiversity International (previously IBPGR or International Board for Plant Genetic Resources) (Engels and Visser, 2006). As judged by the magnitude of the advancements made over the previous decades at the global level, the authors recognized that the response of germplasm banks had been scarce regarding the utilization of the appropriate strategies for the *ex situ* conservation of collections. For curators, this manual constituted a guide for adopting a more critical, balanced and creative approach to germplasm conservation. Useful information was presented on various management aspects to solve frequently encountered operative problems with the incorporation of new and better technologies. In particular, important elements were analyzed and options were discussed to improve the efficiency and effectiveness of operations both according to costs and by taking into account genetic and economic implications for rationalization of the logistics. From a further analysis of the history and evolution of germplasm banks, it was concluded that these banks had gone through periods of questioning about their function or operativity. Among others, the following reasons were given: limited resources; excess or loss of accessions; lack of representativeness of the natural genetic diversity in the accessions, modifications in conservation and multiplication protocols, and changes in the conservation objectives due to the demands of breeding (development of commercial varieties) and agroecological programs (preservation of local varieties or landraces).

More than a decade has gone by since the publication of Engels and Visser´s (2006) document. However, in our opinion, there is still a need to revise the functioning and management of germplasm banks in general. We consider that it is timely to present an approach at the light of principles and methods of Genetics. In this regard, the principles and methods established and used at the individual level (cell, tissue, organ, organism) (e.g., what is the genetic material, how it is transmitted and arranged, how it changes and functions) are not the same as those established and used at the population level (which are related to the course of the genetic material in the populations). We consider that our proposal -based on considerations of the modes of reproduction and their genetic consequences, the genetic structures of wild and cultivated populations, and principles of population genetics- could serve as a base document for discussion among curators, researchers and breeders on the adequacy of the current protocols for *ex situ* conservation of the natural genetic diversity. To the best of our knowledge, this approach relating gametes, gene flow, fertilization and other biological phenomena that have important genetic components has not previously been integrally and routinely used. In this regard, there are many examples in the literature in which the "structure" of collections of wild or cultivated species has been assimilated to the "genetic structure" of populations or in which the term has been used in regard to the total genetic diversity and its partitioning at various levels by means of statistical analysis (ANOVA, AMOVA, STRUCTURE program), even though the definition of "genetic structure" in Genetics is clearly different, as it will be discussed. Moreover, for some statistical analyses (e.g., traditional cluster analysis) it has been considered appropriate to assume that sexual reproduction can occur either by autogamy or allogamy and, therefore, that a population of an autogamous species is genetically homogeneous and a population of an allogamous species is genetically heterogeneous. However, the variability that can be encountered in the genetic structure of a natural population at a given time would depend, among other factors, on the preponderant mode and type of reproduction of the population of origin, as it will be explained.

Ex situ CONSERVATION

Plant germplasm conservation is mainly carried out *ex situ* in the form of samples of propagules (accessions). These propagules can be either the originally sampled ones in natural or naturalized populations, or their regenerants obtained in the same bank or from inter-bank exchange. In the last decades, there has been a change in emphasis away from this type of conservation and towards the *in situ* conservation of locally adapted landraces and crop wild relatives (CWR) within or outside protected areas (Maxted *et al.*, 1997; Maxted *et al.*, 2016; FAO, 2017). However, *ex situ* conservation has advantages and disadvantages *per se* and in relation to other conservation methods (Kjaer *et al.*, 2001, in Hammer and Teklu, 2008); thus, the *ex situ* and *in situ* approaches are complementary, fulfilling different purposes.

Plant accessions are usually conserved under specific categories, mainly assigned according to

morphological phenotypes, with the relatively more recent incorporation of molecular tools (see Camadro, 2012). This type of classification into taxonomic or typological species (TS) responds to the Taxonomic Species Concept (TSC); according to this concept, species are immutable entities because they have reached the end of the evolutive process. Plants can also be classified as biological species (BS) on the basis of breeding relationships when the Biological Species Concept (BSC) is applied, regardless of their morphological phenotypes. TS and BS do not necessary overlap; thus, the use of the term ''species'' generates much confusion when the distinction between them is not clearly made (see Grant, 1981). Moreover, taxonomic categories are periodically subjected to revision because they are human constructions. Thus, taxonomic nomenclatures and "species" numbers in a given plant group can vary over the years according to the taxonomist(s) involved in the task. For example, the number of potato "species" (*Solanum* L. section Potato; Dicotyledoneae) has been reduced in the last 40 years from approximately 235 (seven of them cultivated and 228 wild) to 203, 189 and 111 (four of them cultivated and 107 wild) (in Poulsen Hornum and Camadro, 2021), whereas in brome grasses (*Bromus* L. section *Ceratochloa*), with approximately 160 recognized "species", the large morphological variation encountered in the section led Williams et al. (2011) to point out that "Hybridization is rife in this section, making species boundaries obscure and the taxonomy very difficult". Notwithstanding, and as previously stated, collections are assigned specific categories for their incorporation and conservation as accessions in germplasm banks, without specification of the concept (either TSC or BSC) used for their classification (see an example at http://www.ars-grin.gov/npgs/collections.html). The species concept employed in the taxonomy of a plant group, however, has genetic consequences for both conservation and seed regeneration and multiplication protocols (see Poulsen Hornum and Camadro, 2021).

Germplasm bank accessions can be composed of (a) seeds of sexually reproducing or apomictic plants; (b) plants derived from vegetative organs (e.g., tubers, stolons, corms, leaves) cultivated in the field, or plantlets cultivated *in vitro*; (c) pollen, embryos or tissues conserved in liquid nitrogen (FAO, 2017). This type of conservation is justified when: (a) natural or naturalized populations are subjected to -or at risk of being subjected to- genetic erosion, or are affected by the extinction of native or naturalized plant communities; (b) there is a need for developing or complementing breeding programs through pre-breeding in less domesticated species, or for complementing working collections in breeding programs of advanced-breeding species for transferring genes or gene combinations from unexploited sources; (c) there are lines, clones or compounds synthesizing general adaptation, agronomic aptitude and productive potential that have been discarded in breeding programs, or varieties of reference that have been replaced by new ones in the commercial circuit but that can eventually be of value in breeding; (d) there are landraces or old varieties, often linked with traditional food products and organoleptic properties, that have cultural or economic value (or both) for small farmers.

¿WHAT PART OF THE GENETIC DIVERSITY NEEDS TO BE PROTECTED?

Ex situ conservation steps from the premises that (a) this form of conservation is the safest and cheapest alternative for preserving plant genetic resources for forthcoming generations, and (b) accessions are representative of the diversity encountered in the environments from which they were sampled: spatial (landscape, plant communities), morphological, and molecular. The two premises -along with the provision of detailed passport information- are important. However, an approach is needed to ensure that accessions faithfully represent both the sampled populations and the portion of the genetic diversity that needs to be protected. It has to be taken into account that genetic drift can occur if, in planning the operations, there is not a strict consideration of a combination of various phenomena. These can span from manipulations at the sampling time to various aspects of reproductive genetics during seed regeneration or multiplication, including the possible action of internal crossing barriers within accessions, e.g., male sterility, pollen-pistil incompatibility, nuclear-cytoplasmic genome interactions, among other biological phenomena (see Camadro 2012; Poulsen Hornum and Camadro, 2021). Thus, the estimation of genetic diversity ought to be complemented with detailed information on the genetic structure and reproductive biology of the population at the sampling time and, fundamentally, during the *ex situ* regeneration or multiplication processes. This last concept, if not integrally applied, nullifies the premise of security, economics and representativeness of the accessions because duplicates would not be detected and some gene (allele) frequencies might be unknowingly increased, decreased or eroded during the multiplication process. In summary, the genetic diversity and variability represented by an accession could be unnoticedly changed during propagule regeneration or multiplication; as a consequence, the accession would no longer represent the actual diversity and variability of the sampled population (Hammer and Teklu, 2008; Erazzú *et al.*, 2009; Cadima *et al.*, 2017; Poulsen Hornum and Camadro, 2021).

GERMPLASM BANKS

Many germplasm banks had their origin in plant breeding and research programs and were not necessarily designed to assimilate genetical approaches for *in situ* and *ex situ* conservation. Thus, it is important to critically examine the precise objectives of germplasm banks to identify possible limitations in their functioning. If clear objectives are established, it would be feasible to plan what genetic resources should be conserved and to choose the most adequate protocols for that end, establishing priorities and recognizing limitations and the biological complexities of the species of interest, including the form of propagation., Frankel (1984) proposed to establish core collections to facilitate germplasm management and use after defining the objectives. Core collections are collections of limited size, with minimum similarity among the composing accessions and much smaller than the collection(s) from which they were derived. Or as defined by Johnson and Hodgkin (1999), a core collection is a subset of one collection that represents with minimum repetition the genetic diversity of a cultivated species and its wild relatives.

A CONSERVATION APPROACH BASED ON THE GENETIC STRUCTURE OF POPULATIONS

The main objective of *ex situ* conservation is to have the maximum genetic diversity of a species represented in the accessions, previous establishment of the necessity of conservation, the increment of the number of propagules, and the maintenance of this diversity for conservation and exchange. These aspects ought to be known to define the representativeness of the originally sampled population in the accession. As complements, gaps and priorities have to be identified in the collection for conservation of strategic genetic resources and the determination of their potential applied value.

GENETIC MAKEUP OF POPULATIONS AND INDIVIDUALS IN NATURE

From a biological perspective, a natural population is a community of potentially inter-breeding individuals growing at a given locality, which share a common gene pool and represents a dynamic panmictic unit (Johansen 1903 and Dobzhansky, 1935, in Rieger *et al.*, 1976). The largest group of potentially inter-breeding individuals is the species which, in turn, is composed of local populations, each of them inter-communicating and inter-grading with the others. The sum of all factors governing the pattern by which gametes of various individuals unite with each other during fertilization makes up the population structure which, in nature, is a consequence of gene flow rates and environmental heterogeneity (Gilmoure and Gregor, 1939, in Rieger *et al.*, 1976).

By extension, the genetic structure of a population, either natural or artificial, is the type, quantity and distribution of the genetic variation present in that population expressed in terms of gene (allele) or genotypic frequencies. Thus, the genetic structure of a population depends on the mode and type of reproduction of the plant group or species that conform it. In this regard, it has to be taken into account that higher plants can reproduce either sexually or asexually, or have both types of reproduction available to them; consequently, the genetic structure of a given population can vary over time.

MODES OF REPRODUCTION AND GENETIC CONSEQUENCES

Sexual Reproduction

The production of sexual propagules (sexual seeds) entails the formation of *n* megaspores and *n* microspores (pollen grains or male gametophytes) by meiosis, followed by the formation of *n* female gametes and *n* male gametes by post-meiotic mitosis. The double fertilization of the *n* egg cell and the binucleated (*n* + *n*) central cell of the female gametophyte (embryo sac), each by one of the two *n* male gametes carried by the microspore, originates one 2*n* cell and one 3*n* cell which, respectively, give rise to the 2*n* embryo and the 3*n* endosperm by mitosis (Dumas and Mogensen, 1993). The events involved in sexual reproduction allow for the occurrence of two rounds of genetic recombination: (1) at meiosis, by segregation of chromosomes and genes, and (2) at fertilization, by nuclear fusion of the uniting gametes. Therefore, each sexual cycle provides the opportunity for the formation of new genotypic combinations.

Autogamy and allogamy

There are two types of sexual reproduction: allogamy or cross-fertilization and autogamy or self-fertilization. Allogamy maintains heterozygosity at most loci if the breeding population is large enough, whereas strict self-fertilization leads to homozygosity in most loci and, eventually, to allele fixation.

Two main factors promote allogamy: spatial and temporal separation of sexual organs. Spatial separation can occur (a) within the plant itself, e.g. maize (*Zea mays* L.), which bears female and male inflorescences at different positions along the axis, and (b) between plants, e.g. asparagus (*Asparagus officinalis* L.), with individual plants bearing only one type of imperfect flowers,

either with stamens or pistils (occasionally, perfect flowers are formed in either type of plant, allowing self-fertilization). Temporal separation (dichogamy) is the result of differences in the maturation time of female and male reproductive organs (protogyny and protandry, respectively), which in a plant can occur in (a) flowers or inflorescences along the axis, e.g. maize, or (b) within an inflorescence, e.g. carrot (*Daucus carota* L.) and sunflower (*Helianthus annuus* L.). However, there could be simultaneous maturation (homogamy) without autogamy in the presence of other factors: (a) chasmogamy (the flower is open when pollen is shed and/or the stigma is receptive) in otherwise cleistogamous flowers (the pollen is shed and the stigma is receptive when the flower is closed), e.g. *Bromus* spp. section *Ceratochloa* (Wolff *et al.*, 1996; Langer and Wilson, 1965; Leofanti *et al.* 2013); (b) hercogamy (physiological barriers), in plants with genetically controlled self-incompatibility systems in which the flowers are either (b_1) homomorphic (of one morphological type), e.g. potatoes and tomatoes (*Solanum* L. spp.), stone fruits such as almonds and cherries (*Prunus* L. spp.), Crucifers (*Brassica* L. spp.) such as cabbage, colza and kale, among others, or (b_2) heteromorphic, e.g. common flax (*Linum usitatissimum* L.) and loosestrife (*Lythrium junceum* Banks & Sol.); and (c) sterility (being male sterility the most frequent type) due to malformations in the reproductive organs or abnormalities in meiosis that prevent either production of viable pollen or its release from the anthers and, thus, self-fertilization. Breakdown of hercogamy, dichogamy, or self-incompatibility precedes the shift of the breeding system from obligate outcrossing towards autogamy due to structural and positional changes in the hermaphrodite flower, bud pollination and, finally, cleistogamy (in Frankel and Galun, 1977).

Autogamy and allogamy have both specular positive and negative characteristics. The positive characteristics of autogamy *vs*. allogamy are: genotype fixation and genotype specialization, which result in thriving of adapted genotypes over time in stable environments; guaranteed fertilization with economy of pollen; and adaptation to long distance dispersal because only one seed can start a population. The negative characteristics of autogamy are the other face of the coin: genetic inflexibility due to a lower capacity of "genetic storage" (of alleles and intra-locus and inter-loci interactions) and, thus, inability of the population to cope over time with changing environments ("evolutive compression"); and unguaranteed fertilization with the consequent waste of pollen.

Asexual Reproduction

Asexual propagules can originate by means of (a) seeds (agamospermy) or (b) other structures (agamic or vegetative reproduction). In agamospermy, there could be morphological alternation of generations or not. There is morphological alternation of generations when diplosporous or aposporous 2*n* gametophytes are formed, respectively, from 2*n* archesporial or 2*n* somatic cells, and either the 2*n* egg or other 2*n* cell of the gametophyte develops parthenogenetically in a process accompanied by the development of the endosperm either after fertilization of the central cell (pseudogamy) or without fertilization of this cell. On the other hand, there is no alternation of generations if the 2*n* embryos develop by adventive embryony or sporophytic budding from cells of the nucellus or integuments of the ovule (somatic embryogenesis) (Asker, 1980; Burnham, 1980). In plants with agamospermous reproduction, embryos (a) can be clones of the mother plant if they originate by somatic embryogenesis, apospory, or diplospory with a modified meiosis genetically equivalent to a mitosis, or (b) can genetically differ from the mother plant if the modified meiosis in diplospory entails a certain amount of recombination. In plants with agamic or vegetative reproduction, propagules (bulbs, corms, tubers, stolons, or rhizomes, among other structures) are formed by mitosis in somatic tissues, thus, they are clones of the mother plant.

ARE THE MODES AND TYPES OF REPRODUCTION STRICT?

Higher plants may have more than one mode or type of reproduction as a result of genotype x environment interactions. Sexually reproducing plants can be (a) autogamous, e.g. wheat (*Triticum aestivum* L.), tobacco (*Nicotiana tabacum* L.), garden tomato (*Solanum lycopersicum* L.); allogamous, e.g. maize, carrot, garden asparagus; (b) autogamous with a percentage of allogamy, e.g. beans (*Phaseolus* L. spp.); (c) allogamous with a percentage of autogamy, e.g. maize, sunflower (*Helianthus annuus* L.), asparagus. Autogamous plants could be considered a prelude to evolutionary extinction if it were not for the fact that local differentiation in ecological niches maintains a massive storage of genetic diversity (in Frankel and Galun, 1977). Similarly, asexual reproduction is not strict; otherwise, it will also be an end road in evolution. It is frequently combined with sexual reproduction by allogamy, e.g. potatoes, grasses.

NATURAL AND NATURALIZED POPULATIONS

Sexually Reproducing Species

In autogamous species, individual plants with disomic inheritance (diploids and disomic polyploids, e.g. 2x *Triticum monococcum* L., 4x *T. turgidum* L., 6x *T.*

aestivum L.) are expected to be highly homozygous for one genetic combination (Fig. 1a) or more than one (Fig. 1b). Populations of autogamous species, however, can be genetically homogeneous to a greater or lesser extent depending on whether they have a percentage of allogamy or not. For example, the percentage of allogamy in Proso millet (*Panicum miliacium* L.), with wind-dispersed pollen, can be more that 10%, whereas in Lima beans (*Phaseolus lunatus* L.), with bees-dispersed pollen, this percentage can range from 0% to 80%. Moreover, the proportion of cleistogamous *vs.* chasmogamous flowers (e.g., in *Lespedeza* Michx. ssp.) could variably increase the percentage of allogamy in a given season (in Frankel and Galun, 1977). If individual plants have opportunities for hybridization even from time to time, the population can be composed of plants either homozygous for one genetic combination (Fig. 1a) or more than one (Fig. 1b), or heterozygous for one or more loci (Fig. 1c) because they might be F_1 hybrids, backcrosses to the homozygous parents, or advanced segregating generations. Therefore, populations can be either homogeneous or heterogeneous in various degrees. In inbreeding species, the variation among populations is expected to be larger than within populations in contrast with outbreeding species. In a review of experiments carried out with isozymes in autogamous and allogamous species, Schoen and Brown (1991) found that inbreeders exhibited markedly greater population variation than outbreeders according to Nei´s gene diversity statistics.

On the other hand, allogamy is obligate only in monoecious species with strict self-incompatibility systems, and in dioecious species. The spatial and temporal separation of the reproductive organs, as previously explained, promotes but does not force this type of sexual reproduction. In individual plants of both diploid and polyploid allogamous species, most loci are expected to be in heterozygosity, although there could also be loci in homozygosity. Natural populations are expected to be highly heterogeneous (Fig. 1d), being the genetic diversity higher within than between populations as demonstrated, for example, in wild potatoes (Bedonni and Camadro, 2009; Erazzú *et al*. 2009).

Asexually reproducing species

A few higher plants exhibit only asexual reproduction (e.g. garlic, *Allium sativum* L.) but most plants with this mode of reproduction can also reproduce sexually under certain environmental conditions (see Frankel and Galun, 1977). The environmental conditions can modify not only the proportion of allogamy in sexual reproducing plants, as previously explained, but the preponderant mode of reproduction of a given population as well. Examples can be found in apomictic grasses (Knox, 1967; Quarin, 1986; Rebozzi *et al*., 2011) and wild potatoes (Leofanti *et al*., 2019), among other plant groups. It is a common mistake to consider that natural populations of asexually reproducing plants are genetically homogeneous. On the contrary, these populations can be composed of plants of either the same genotype (one clone; Fig. 1e and 1g) or different genotypes (more than one clone; Fig. 1f and 1h) because asexual reproduction is usually combined with sexual reproduction by allogamy. Therefore, a population with the two alternative modes of reproduction can be a mix of clones as a result of either hybridization followed by vegetative reproduction in the subsequent generations or facultative apomixis. Individual plants of asexually reproducing species can be highly heterozygous, but some loci can be in homozygosis. Populations with asexual reproduction can be either homogeneous or heterogeneous in various degrees (see Ellstrand and Roose, 1987).

Summarizing, a thorough knowledge and understanding of the reproductive biology and genetics of the species of interest is needed in order to (1) develop the appropriate sampling and regeneration protocols to try to capture an important amount of the genetic diversity present in a population, and (2) avoid or minimize gene (allele) erosion during seed regeneration. Moreover, and given that the types and modes of reproduction are not necessarily strict in a given plant group and a given environment, it is: (a) inappropriate to carry out statistical analyses under the assumption that populations have only one type of reproduction (e.g. for sexually reproducing species, either autogamy or allogamy) and, therefore, that they there are genetically either homogeneous or heterogeneous, and (b) advisable to resample the populations in environmentally contrasting years, whenever possible. In this regard, samples of a given population taken in different moments should be used to conform the accession (instead of naming each sample as a new accession) to maximize the amount of the captured natural genetic diversity at a given site. It is our opinion that no specific guidelines should be given for curators. Instead, and based on the knowledge of the reproductive biology and genetics of the plant species or group of interest, the principles and methods of population genetics should be applied to prevent or reduce gene erosion in the conserved germplasm.

BREEDING POPULATIONS

Genetic makeup

Rimieri (2017) has pointed out that it is necessary to differentiate *ex situ* and *in situ* conserved plant genetic resources from those plant resources collected, maintained and utilized for human subsistence, which

are the result of the application of selection or breeding methods. According to this approach, the protection of the biodiversity and the application of mutagenic, biochemical, molecular and genetic engineering tools are compatible and complementary.

Plant breeding is the heritable improvement of plants, usually acknowledged as a combination of art and science. Approximately 11,000 years ago, domestication of plants and animals evolved from the hunter-gatherer lifestyle. But it was in the 20th century, with the rediscovery of Mendel´s laws of inheritance, that plant breeding became an applied discipline, which makes use of principles from a variety of other disciplines to improve the genetic potential of plants cultivated for food, feed, and/or metabolites of interest, among others. Plant breeders make use of conventional methods (parental selection, controlled crosses, progeny selection) to introduce desirable traits to their object of improvement (Gallais, 1990; Allard, 1999) with the relatively more recent aid of biotechnologies, e.g., transgenesis, cisgenesis, intragenesis, and gene edition (Al-Khayri *et al*. 2015; Cardi, 2016). In spite of the advancements in genome manipulation, plant breeding remains a high time- and resource-consuming process, particularly in crop species with narrow genetic bases.

The final products of plant breeding are cultivated varieties or *cultivars* (a term coined by contracting the two previous terms to establish a difference with *botanical varieties*, which correspond to a taxonomic rank between subspecies and form). Cultivars are obtained in usually long processes, essentially Mendelian in nature and probabilistic. They are classified into five types according to the reproductive system of the target species and the genetic structure of the artificial populations: (1) *lines* or *line cultivar*, generally of only one genotype (*pure line*; Fig. 1a); (2) F_1 *hybrid* or *hybrid cultivar*, of only one genotype resulting from a cross between two pure lines, with heterotic effects, represented in Fig. 1e with two loci in heterozygosity (hybrid vigor) and one locus in homozygosity (overdominance), and in Fig. 1g with three loci in heterozygosity; variants of this type of cultivar are named *semi-hybrid cultivars*; (3*) population or population cultivar*, a mixture of genotypes of either autogamous (Fig. 1b), allogamous (Fig. 1d), or apomictic plants. In forage crops, a population cultivar composed of practically isogenic pure lines, similar in phenology and morphological type, is known as a *multiline cultivar*; in allogamous species, this type of cultivar is a population of wide genetic base resulting, in general, from mass selection (Gallais and Bannerot, 1992); (4) *synthetics* or *synthetic cultivar*, similar to population cultivars but only for allogamous species, with paternal control of the origin (*polycross*) (Fig. 1d), or *hybrids* with low vigor depression in F_2; (5) *clones* or *clone cultivar*, composed of only one genotype (Fig. 1e), or two or more genotypes, e.g., clonal hybrids of dioecious species such as asparagus (Fig. 1f) and scions and grafts of fruit trees and ornamentals (Fig. 1h), selected from any structure or obtained by mutagenesis and either macro- or micropropagated (Rimieri, 2017). The subject of the plant protection system -that will be further explained- is a variety (cultivar), that is, a plant grouping within a single botanical taxon of the lowest known rank. Such grouping is defined by the expression of the characteristics resulting from either a given genotype (e.g. one clone, line, or F_1 hybrid) or a combination of genotypes (e.g., a complex hybrid or synthetic variety) (UPOV, 2002).

INTELLECTUAL RIGHTS PROPERTY

The conservation and utilization of plant genetic resources have always required the consideration of diverse factors beyond the biological diversity itself. Among others, the following can be mentioned: genetic transformation technologies, technologies of information and communication (TICs), linked to an increasing world recognition of the value of these resources (Visser and Nap, 2002), and intellectual rights property of both genetic resources and breeding products (Gepts, 2006).

The International Union for the Protection of New Varieties of Plants (UPOV) was created in 1961 to provide and promote an effective system of plant variety protection, with the objective of encouraging the development new plant varieties in its numerous member countries (UPOV, 2020). However, with the advent of plant biotechnologies, patent rights began to affect the access to both genetic resources and commercial varieties. In contrast to the breeder´s rights, patent rights limit the access of third parties to patented genes, with the consequent negative effect on the use of genetic resources. As Eriksson et al. (2020) have discussed, different legal frameworks applicable to the use of the genetic resources have been developed. With the scientific and technical progress in research and breeding achieved in the past few decades, these frameworks have become increasingly complex. Notwithstanding, the Convention on Biological Diversity (CBD, 2020) in its art. 13, recognizes the sovereign rights of the states on the genetic resources located within their frontiers. Based on the principles contained in the CBD and the 2011 Nagoya Protocol plus the decisions of the Parties, international goals on access and benefit-sharing have been established (see Sirakaya, 2019).

UPOV is only concerned with protected plant varieties. However, there is a spectrum of plant genetic resources that does not fall into this category: populations of CWR, landraces, and unprotected plant varieties. These genetic resources are not affected by UPOV or plant breeders´ rights, but they may be regulated by other

treaties or schemes, e.g., the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRF), the previously mentioned CBD, and seed marketing regulations (UPOV, 2016).

FROM GERMPLASM BANK ACCESSIONS TO COMMERCIAL CULTIVARS

The potential utilization of *ex situ* conserved germplasm responds to specific needs of broadening the genetic variability or the gene pool of the breeders´ working collections, particularly in crop species in which the advancements by selection are slow. From this germplasm, new genotypes or gene combinations can be developed for incorporation into breeding programs (Cooper *et al.*, 2002; Rimieri and Wolff, 2010).

One proposal to combine a more efficient conservation of the genetic diversity present in the accessions and to utilize part of the genetic variability of this germplasm in plant breeding is the development of the previously mentioned core collections. The establishment of core collections, which concentrate high genetic diversity in a small number of samples with the avoidance of duplicates, can contribute to the utilization of germplasm in research and pre-breeding, and to the increase of the efficiency of germplasm bank management and inter-bank exchange. Furthermore, with the complement of molecular biology tools, genetic engineering and geographic information systems (GIS), the efficiency and sustainable conservation of plant genetic resources advocated by FAO (1996) would be likely incremented.

GENETIC RESOURCES, POPULATION STRUCTURE AND OBTAINMENT OF COMMERCIAL CULTIVARS

The expansion of the genetic base and pre-breeding shortens the gap between basic germplasm and crop genotypes. However, plant breeders seem to be reluctant to employ plant materials coming directly from germplasm banks because these materials lack, in general, adaptation for their use in breeding. The lack of adaptation is a consequence of the cultivation environment of the crop species and the agronomic management practices, plus the genetic structures of commercial cultivars and the compatibility and interactions of the wild germplasm with the genetic background of the breeder´s elite collection. Notwithstanding, the three elements -genetic resources, population structure, and commercial cultivar development- can be combined. Following, an example is given of forage breeding program to illustrate the close inter-disciplinary relationship between the use of germplasm from working collections and germplasm banks and the application of methods and tools of commercial cultivar development.

In forage crops in general, cultivars are populations, lines and genotypes adapted to the environmental and agronomic conditions of a growing region. They may have their origin in one or more of the following: (a) working collections of research groups involved in population evaluation and selection, (b) foreign cultivars, (c) cultivars adapted to cultivation conditions and animal utilization but no longer available in the market, (d) breeders´ own collections obtained from native and naturalized populations or from old implanted fields, and (e) selected samples -according to previously defined criteria- from national and international collections of botanical gardens, introduction and acclimatization gardens, and germplasm banks. It is, therefore, necessary to remark that the decision on the germplasm to be conserved and its possible utilization in breeding programs has to be based on (1) the initial germplasm, obtained by collection or exchange, with special emphasis in its representativeness of the genetic diversity of the species and the adaptation to the environment and cultivation; and (2) consideration of (a) agronomic and genetic parameters in the original samples and in the subsequent characterization, (b) the predominant mode of reproduction, for propagule multiplication, and (c) the predominant or more representative genetic structures, also for propagule multiplication or the development of core collections, pre-breeding, or commercial cultivar breeding.

TALL FESCUE AS AN EXAMPLE

Tall fescue (*Festuca arundinacea* Schreb.) is a perennial forage grass of temperate climate, of utmost importance and diffusion in Argentina. This species is allogamous, with cleistogamous and chasmogamous flowers, of hexaploid origin and with disomic inheritance. The breeding program carried out at the Pergamino Experimental Station (Exp. Stn.), National Institute of Agropecuarian Technology (INTA), in the Pampas region of central Argentina, is succinctly described in Table 1. It is proposed as an integral model for germplasm management and utilization in general.

The needs of initiating a tall fescue breeding program and of creating a forage germplasm bank in the country stepped from the following:

(1) agroecological conditions: (a) there were no native forage species adapted to cattle grazing, and (b) the forage production of native and naturalized forage species subjected to intensive grazing was low.

(2) technological situation: (a) there were no forage germplasm banks, and (b) the grasslands were subjected to intensive grazing.

In response to this situation:

(1) Temperate forages species with high forage production and adapted to intensive grazing were introduced, characterized and evaluated in agronomic, biological, genetical and animal production studies.

(2) Populations and ecotypes for planting and grazing were selected; cultivars were created, released and disseminated in the region (the area of cultivated pastures was increased with the local cultivar *Pergamino El Palenque MAG*); adaptation and production were evaluated. This germplasm became part of both the working collection of the forage breeding program and the germplasm bank of Pergamino Exp. Stn.

(3) Foreign cultivars were introduced to widen the genetic base of tall fescue in Argentina but, in general, they had poor agroecological and grazing adaptation.

(4) The need of exploring the available global germplasm was established. A forage germplasm bank was created with the adapted local germplasm and the world collection. Collections were evaluated and characterized; protocols were applied to maintain the genetic diversity; core collections were created.

(5) Pre-breeding was initiated for other traits (adaptation to saline soils, forage nutritional value, etc.).

(6) Selected genotypes continued to be incorporated into the germplasm bank.

(7) Animal production was increased in the region.

Summarizing, steps and protocols were followed in tall fescue to integrate objectives of introduction of forage species for intensive grazing, obtainment of populations and ecotypes for germplasm management and utilization in integrated crop-livestock systems, adoption of modern cultivars, pre-breeding for other traits, enhancement of the germplasm bank and increase of animal production. We consider that the Argentinian tall fescue breeding program is a good example of FAO´s proposition (FAO, 1996) on the association and complementation of germplasm banks with breeding programs.

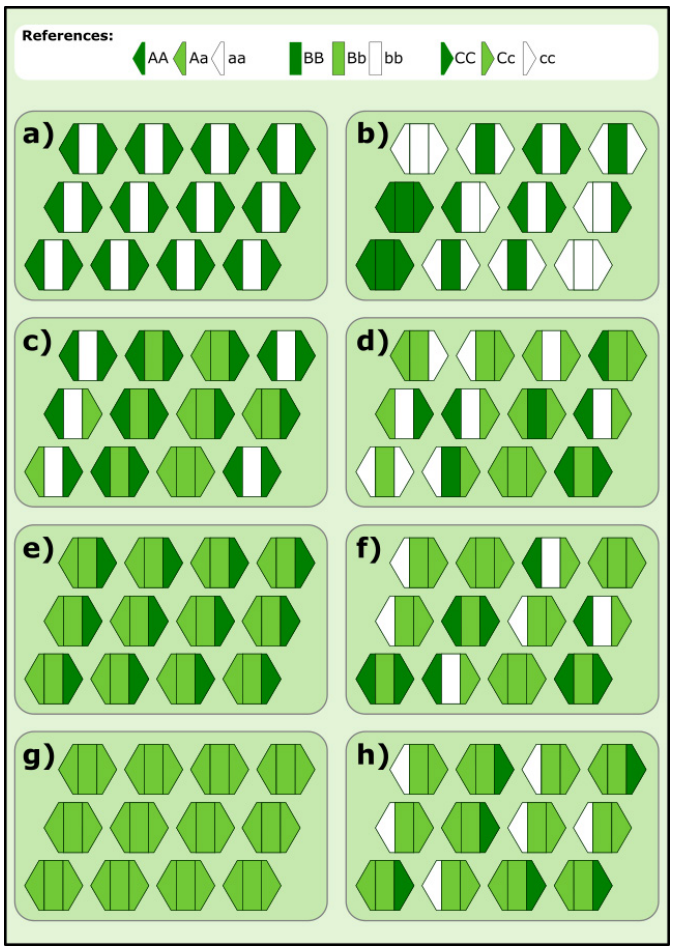

Figure 1. Genetic structure of natural *(NP)* and breeding *(BP)* populations according to modes and types of reproduction. *NP*: (a), (b) and (c) *autogamous diploids and disomic polyploids* (a) homogeneous, with all loci in homozygosity in one combination, (b) heterogeneous, with all loci in homozygosity in various combinations, (c) with a percentage of allogamy; (d) *allogamous diploids*, heterogeneous, with loci in homozygosity and heterozygosity; (e) to (h) *clones*, homogeneous, with either loci in homozygosity and heterozygosity (e) or all loci in heterozygosity (g) for one combination, or heterogeneous with more than one genotype (f) and (h). *BP*: *lines*, homogeneous, with all loci in homozygosity (a); F_1 *hybrids*, homogeneous, with two loci in heterozygosity (hybrid vigor) and one in homozygosity (overdominance) (e) and with all loci in heterozygosity (g); *populations*, heterogeneous, of autogamous (b) and allogamous (d) species; *synthetics*, heterogeneous, with loci in homozygosity and heterozygosity (d); *clones*, homogeneous with loci in homozygosity and heterozygosity in one combination (e) and (g), or heterogeneous, with loci in homozygosity or heterozygosity in more than one combination (f) and (h).

Table 1. Methods and achievements in the Argentinian tall fescue (*Festuca arundinacea* Schreb.) breeding program: from germplasm introduction and collection to obtainment of commercial cultivars.

Year	Institution	Methodology	Achievement	References
1940-	Pergamino Exp. Farm, Ministry of Agriculture (MAG)	Introduction of forage crop populations Studies of adaptation to edafoclimatic environments of the Pampas	Establishment of introduction and acclimatization gardens	Boelcke and Echeverría (1950)
1951-		Evaluation of populations, followed by 1-2 selection cycles or off-type plants roguing	Obtainment of phenotypically uniform populations according to species. Release of the first 38 Argentinian cultivars of 28 forage species, with wide adaptation and diffusion, validated over the next 30 years	Villar and Serrano (1963) Serrano (1985)
***ca*.1961**		Mass selection in introduced germplasm: Alta, Kentucky 31, Goar (records kept on materials and trials)	Release and diffusion of cultivar-population* *Pergamino El Palenque MAG* (being free of *Acremonium coenophialum*, it became a reference cultivar)	Maddaloni and Ferrari (2001)
1980-	Pergamino Exp. Stn. National Institute of Agropecuarian Technology (INTA)	Organization of a Forage Germplasm Bank	Forage Germplasm Bank established. Incorporation of (a) populations introduced from 1947 and on, selected for adaptation and persistence, (b) samples (accessions) from sown and naturalized Pampas populations	

Table 1 (continue). Methods and achievements in the Argentinian tall fescue (*Festuca arundinacea* Schreb.) breeding program: from germplasm introduction and collection to obtainment of commercial cultivars.

Year	Institution	Methodology	Achievement	References
1990-		Selection with methodologies according to demands of a competitive cultivar market. Complementary germplasm studies	Initiation of (a) a breeding program for obtainment of superior synthetic cultivars, (b) characterization of accessions	
1995-		Obtainment of the first Argentinian synthetic cultivar** *Palenque Plus INTA*		Rimieri (1995)
		120 derived genotypes selected and evaluated		
2000-		Incorporation of a large part of the fescue world collection	350 accessions introduced	Rosso *et al.* (2001)
		Morphological and agronomic characterization	36 selected populations	Rimieri andWolff (2010)
		Evaluation for forage nutritive value		
		Molecular (SSR) characterization	Core collection established	Cuyeu *et al.* (2013)

CONCLUSIONS

The premises of this paper are that *ex situ* conservation of the genetic diversity contained in CWR and the utilization of the natural genetic variability in cultivar breeding require the application of reproduction and population genetics concepts in order to choose or develop the appropriate criteria and experimental strategies.

An important fact that needs to be taken into consideration for devising germplasm collection and *ex situ* conservation strategies is that the modes and types of reproduction have different genetic consequences for the following generation. Natural or naturalized populations, even those of autogamous species, can be heterogeneous, and the predominant mode and type of reproduction of a given species can vary according to environmental conditions during the growing cycle.

Biological systems, particularly plant systems, are very complex, thus, assumptions are usually made in an attempt to investigate them. Since discrepancies between "reality" and "assumptions" can be large, the conclusions withdrawn from experimental works need to be adjusted to the plant materials and methods of study to have scientific support. In this regard, there are many reports in the literature on plant and crop physiology of the main food crops (e.g., wheat, maize, sunflower, soybeans) and the "genetic progress" or "genetic gain" that has been achieved in commercial cultivar breeding over the past decades (see Lo Valvo et al. 2018 as an example). However, their potential contribution in crop breeding needs to be ascertained by making focus on the analysis of the genetic structure of populations and the sources of genetic variability available to the breeder (commercial cultivars, land races, CWR). The genetic structure has to be related to the main methods used in those studies and others of related disciplines for the interpretation of the results in the frame of their eventual application in crop management or breeding.

PROPOSAL

We consider that the following information is needed as a basic input to start the analysis of the current germplasm bank protocols at the light of the principles and methods of Genetics:

(a) Genus (or genera) and species of accessions in the germplasm bank

(b) Preponderant mode(s) and type(s) of reproduction

(c) Geographic distribution and sampled areas

(d) Sampling strategies

(e) Passport data of collections in general, from the oldest to the newest

(f) *Ex situ* regeneration/multiplication protocols

(g) Characterization type (morphological, genetic, molecular, agronomic), if any.

This information would allow the evaluation in the *ex situ* collections of:

(a) Representativeness of the collections, geographical and environmental (at macro- and micro- levels).

(b) Adequacy of strategies and protocols for collection and regeneration or multiplication of accessions to the principles of population genetics: population reproductive size (*N*= actual number of plants in the population, and *Ne*= effective number of plants, which contribute alleles to the next generation), population genetic structure, gene (allele) frequencies, processes that can alter gene frequencies.

(c) Representativeness of the natural genetic diversity in the collections.

(d) Necessity of carrying out new collections in the already sampled areas or in as yet unexplored ones.

Furthermore, to ascertain if wild germplasm conservation and commercial breeding converge at some point, the following questions should be addressed:

(1) In pre-breeding:

(a) Is pre-breeding an objective of germplasm banks?

(b) What is considered to be more important in the germplasm bank, the representativeness of the natural genetic diversity in the accessions or the likely immediate use of the conserved germplasm?

(2) In breeding:

(a) Is it considered that the collections can be directly used in breeding programs or that pre-breeding is required as a first step?

(b) Is it known which is the genetic background of populations or genotypes adapted to cultivation that has to be maintained or recovered after manipulations to incorporate new germplasm in the cultivated pool (e. g. hybridizations, backcrosses or other techniques or methods)?

As a first step in this direction, we will coordinate a workshop which is part of the program of ALAG 2021 (XVIII Latin American Congress of Genetics; alagenet.org/alag2021/en/scientific-program/#talleres). In advance, the invited researchers and curators will provide in written response to the formulated questions. The discussion and analysis of the responses will be carried out at the light of the principles and methods of Genetics during the event. The expected final product is a document on the current managing practices in germplasm banks of seven participating countries; if appropriate, the document will also contain propositions for the eventual modifications of protocols.

Finally, as Maxted and Kell (2009) have pointed out, there is a need for CWR characterization and evaluation, development of genomic databases of known useful genes from these sources, and improvement of gene transfer techniques from wild to cultivated species, among others. Notwithstanding, we consider that a previous basic requirement for successful conservation and utilization of the natural genetic diversity and genetic variability is the application of strategies and protocols based on the principles and methods of population genetics, modes of reproduction and genetic structures of CWR populations.

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