APEC-ATCWG Workshop (2008)
Capacity Building for Risk Management Systems on Genetic Resources
79~100

Enhanced Conservation and Management of Canadian Bioresources

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Abstract

An economically viable, environmentally sustainable, secure and safe agricultural system is dependent on the availability of a genetically diverse assemblage of plant, farm animal and microbial bioresources that is conserved, managed and utilized appropriately. Genetic diversity is raw material for selection and improvement in productivity and quality, resistance to pests and diseases, adaptability to changing and adverse growing conditions, materials for bio-based products, and contributes to the creation of a safe and secure food system. However, the genetic diversity of Canadian bioresources, like many others in the world is threatened by genetic erosion of crop and livestock gene pools, habitat loss, and for social and economic reasons[1]. The intent is to present various conservation and management practices which Canada has implemented to reduce the risk of genetic loss of crop and livestock gene pools with emphasis on genebanks.

Introduction

Authoritative knowledge of our bioresources, and the improvement and development of new techniques for conservation, management and utilization are ongoing challenges. The maintenance of genetic diversity requires appropriate conservation techniques, but for many species knowledge of optimal protocols is limited or unknown. The reproductive biology of many native Canadian plant species (including the wild relatives of major crops and many underutilized species) is poorly understood, making the development of regeneration procedures difficult. Conservation of fruit and potato clonal germplasm requires

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optimization of *in vitro* culture techniques, and virus indexing or heat therapy to obtain clones free of infection [2,3,4]. Knowledge of cryopreservation techniques and successful methods of regeneration for many animal/poultry species are largely lacking [5]. Understanding the molecular mechanism behind apoptosis in bull sperm may enhance survival in the female reproductive tract and increase the fertilization rate [6]. Cryopreservation of swine and avian semen has not been optimized in terms of suitable cryoprotectants, or freezing rate [7]. Vitrification, a form of cryopreservation, has not been optimized for bovine oocytes and embryos. Progress has been made in the last decade in the production of in vitro pig embryos, maturation of oocytes, fertilization, and culture including non-surgical embryo transfer, but much remains to be done [8,9,10,11]. Conservation of viral and fungal collections is challenged by a lack of knowledge of optimum storage protocols, regeneration via live hosts, the maintenance of correctly identified, pure cultures, and the development of new diagnostic tools to verify virus identities [12]. Correct identifications of both fungi and viruses are a necessity for regulatory agencies concerned with quarantine and bioterrorism issues, disease resistance studies, diagnostics research, and reference strains for indoor air quality companies, and screening programs for novel pharmaceutical compounds[13]. The characterization and evaluation of bioresource attributes enables the development of new information about, and/or identification of, new sources of plant disease resistance, improved nutritional quality and bioactive compounds. Plant germplasm with genes conferring resistance to diseases reduces the need for pesticides and contributes to environmental health [14]. A few sources of resistance to ascochyta blight in lentil and chickpea have been identified and incorporated into breeding programs, and resistant cultivars have been released. Germplasm with different sources of resistance needs to be acquired and combined to provide resistance against highly variable pathogens [15,16]. Additionally, resistant germplasm is required for cultivars where no resistance is available, or where resistance is at risk of breaking down. Many plant species native to Canada are related to crop plants, and a significantly greater proportion may have economic value [17,18,19]. Native species are sources of secondary metabolites with biological activity which may lead to new herbal, pharmaceutical or insecticidal products [20,21,22]. Compounds derived from underutilized crucifer crops such as *Camelina* and *Crambe* have wide applicability for both industrial and food uses. Wild crucifers are also a novel source of important traits for canola; eg. cytoplasmic and nuclear male-sterility inducing systems critical for the F_1 hybrid seed industry, drought tolerance, and disease and insect resistance[23,24]. Applications of molecular markers in the management of genetic resources have been successfully made to assess

the extent and distribution of genetic diversity and to evaluate genetic relationships of genebank accessions[25,26]. These applications have helped resolve issues such as accession identification and duplication [27]. Molecular diversity investigations of rare or endangered native plant species are few. Knowledge of the genetic diversity and structure, mating system and gene flow of native species is essential for monitoring their genetic status in natural populations and for the development of effective *in situ* conservation strategies [28]. The Germplasm Resources Information Network-Canada (GRIN-CA) was created to manage the information generated in the Canadian germplasm network. The challenges for future GRIN-CA development will be to maintain good communication between system developers and users, to keep the system flexible and adaptable to change, and to follow international standards. ORACLE system software is complex but flexible, and requires a considerable amount of system management, upgrading, administration and monitoring. Ongoing acquisition, storage and regeneration of germplasm including barley, oat, wheat, oilseed and pulse crops are strategically important for Canada [29,30,31,32]. Not a single major domesticated crop has either its centre of origin or domestication in Canada, and crop development will continue to depend on introduction of foreign and other diverse germplasm. Similarly, the maintenance and use of genetic diversity in Canadian farm animals requires ongoing acquisition and proper evaluations of genetic diversity within and among breeds [33,34,35,36,37].

Mandate

The mandate for the Canadian Genetic Resources Program is to conserve, preserve, enhance and increase utilization of plants, animals, microbes and plant viruses of economic importance to Canada. This mandate includes wild species including native Canadian plant species of economic importance to Canada. This mandate probably differs little from that of most other national genetic resource programs in the world. Canada's national program constantly keeps in mind methods and policies to reduce risk to collections, facilities and training to enhance capacity and what clients of the program require from it: quality germplasm for breeding and research and relevant information about the germplasm and about the genetic diversity within the bioresource.

Facilities

Adequate facilities (buildings and field plots) play a key role in the conservation of genetic resources. Facilities impact many of the basic functions conducted while maintaining

and conducting research on the germplasm conserved, in reducing risk to collections and in building national and international capacity. Security of collections is of national importance and can also play a significant role in health and safety for staff. Canada has implemented a number of security measures to ensure the safety of the facilities, the collections and for staff. Some of these are:

1. use of active and passive methods (boulders, trees, external lights) to reduce physical harm to buildings;
2. use of restricted access to buildings and seed storage vaults;
3. secure fencing around all field plots with restricted access to plot areas;
4. use of cameras at all entrances, within seed storage vaults and cryogenic rooms;
5. internal fire suppression systems (not water, but compounds (FM-200) which displace oxygen) especially in the seed storage vaults;
6. “hot buttons” which when pushed announce a major problem and call for assistance within the building and local security forces in seed storage vaults and cryogenic rooms; and
7. oxygen monitors and air replacement in cryogenic rooms, slight negative pressure.

Acquisition

Genetically diverse collections are the foundation for any national program and take considerable time, effort and planning to accumulate so as to constantly meet current and future national needs. The needs of a country will vary over time and may be domestically or internationally focused. In Canada initial plant acquisitions focused on a few important genera: *Medicago, Lycopersicum*, *Triticum*, *Avena*, *Hordeum*. Additions to collections occurred through extensive expeditions to the Mediterranean region and through exchange with other countries. Policy agreements with international agencies (ICPBR) influenced the Canadian collection and resulted in Canada accepting world responsibility for base collections of *Hordeum* and *Avena* and duplicate back up collections of Crucifers and *Pennisetum*. At one time acquisition criteria for each major crop group were established, but these proved to be too inclusive and failed to work (too little was excluded). Recently, the plant germplasm program is focusing on new acquisitions to address specific crop production concerns such as climate change (germplasm with specific stress traits (heat/drought tolerance, disease resistance) and native Canadian plants from costal areas (flooding) or high altitudes, bioproduct traits, newly registered Canadian germplasm (cultivars), and germplasm to increase the genetic diversity (genes/locations) of economic

importance including Canadian species at risk. Applying GIS technologies to identify gaps in collections, to understand the ecogeographic distribution of germplasm and to link these variables to observed genetic variation (using molecular techniques) should now be emphasized. The technologies may help us to better understand why the variation exists. The Canadian animal program is focused on acquiring only semen, embryos and suitable DNA samples (eg. blood, hair) of domestic breeds including the more than 100 breeds at risk in Canada. Samples from animals entering domestication (buffalo, elk, and deer) are also being acquired when available. Accessions in the animal program use a specific donor agreement outlining obligations including passport information.

Maintenance

Germplasm (seed) is ideally maintained in either mid-term storage (working collection) at +4°C and 20% RH or in long-term storage (base collection) at –20°C. A backup electrical generator and compressors exist for cold storage rooms. Clonal germplasm (potato, fruit tree and small fruits) is maintained in orchards, screenhouses, greenhouses or *in vitro*. Animal, microbial and virus germplasm is keep in cryogenic facilities in liquid nitrogen at –196°C or some fungi are kept as dry spores. Considerable effort has been extended to reduce risk to collections including the following: for seed, safe secure duplicate backup of samples at remote locations from Saskatoon, either with the USDA National Genetic Resources Unit, Fort Collins, CO or the Svalbard Global Crop Diversity Trust seed vault, Norway; for clonal material from within Canada backup takes place at Saskatoon, when possible. To reduce risk of loss, many clonal genebanks seek alternatives to duplicate local orchard plantings for backing-up their collections, eg., via developing cryopreservation or *in vitro* maintenance or by establishing duplicate orchard plantings at other sites. Unfortunately research for developing techniques for conserving clonal crops from seed appear lacking. Although this approach may not be feasible or biologically sound (recalcitrant versus orthodox seeds, genetic integrity issues) for some crops, it may be possible for other species or populations.

Regeneration with emphasis on cross-pollinated crops

Reasons for regeneration include: 1. low number of seeds due to: frequent request of a sample (distribution requirement); a sample entering the collection with an initial low number of seeds; the need to multiply seed stocks to meet recommended quantities for base collections; or the seeds have been identified as forming a minor part of another accession

and have to be treated separately. 2. Reduced viability due to natural aging during storage or to low quality of the original seeds. Even under optimal *ex situ* storage conditions, viability declines and the result is a loss of both genes and genotypes. In the case of unique material, such losses may be irreplaceable. Therefore, the monitoring of viability and timely regeneration must be a priority activity for any genebank to reduce risk of genetic erosion. In general, a viability of 85% is considered the minimum to avoid genetic erosion within an accession [1].

Cross-pollinated species appear to have little effect on the world's food supply – possibly no more than 1%. Important foods such as grains, potato, sweet potato, cassava, banana and coconut tend to be wind pollinated, self-pollinated, and partially insect-pollinated or are propagated asexually or develop parthenocarpically. However, when total animal and plant products are considered, about 1/3 of the human food diet is dependent, directly or indirectly, on insect-pollinated plants. Animal food products such as beef, pork, poultry, lamb, milk and cheese contribute about half of the diet. These products are derived, in part, from insect-pollinated legumes such as alfalfa, clover, or trefoil. More than half of the world's diet of fats and oils come from oilseeds- canola, coconut, cotton, oil palm, olive, peanut, soybean and sunflower [38]. Many of these plants depend on or benefit from insect pollination. Regenerating cross-pollinated crop species is particularly challenging in gene banks for a number of reasons.

Lack of information

General patterns of pollen transfer within and between flowers and plants are well known [39,40]. Terms such as cleistogamy, autogamy (selfing), allogamy, xenogamy (crossing) illustrate the diverse array of methods plants manipulate breeding systems and control genetic structure of populations and patterns of evolution. Breeding systems are under genetic control and can be selected for. They are rarely fixed and static, but are fluid and respond to selection pressures in a variety of subtle and interrelated ways. As breeding systems control genetic variability, and are themselves controlled by components of genetic variability, they are subject to feedback positive, negative or stabilizing [39]. Part of the challenge is that most seed plants have a wide range of reproductive options available for selection. These may arise constantly, through genetic variation, or intermittently, through mutation. Broadly speaking these are [39]: hermaphroditic versus unisexual; self-pollination

versus cross-pollination; self-fertilization versus cross-fertilization; and sexuality versus asexuality.

Detailed pollination requirements for the major cultivated crop species are known [41,42,39,43]. However, the reproductive biology for many crop species (including wild relatives of major crops, underutilized or minor crops) is not yet sufficiently understood, making the development of regeneration protocols difficult and placing them at risk to genetic erosion. This matter requires urgent research.

Comparative studies among species within a genus provide valuable insights into the variability of reproductive systems. Estimates of the amount of self- or cross-pollination and self-incompatibility can be determined using 8 treatments involving bagged or unbagged and emasculated or untreated flowers [40]. The rate of self-pollination can be estimated by comparison of the values of seeds from naturally pollinated flowers with those of hand self-pollinated and hand cross-pollinated. The selfing rate, S can be estimated following Charlesworth and Charlesworth [44]:

$$S = \frac{P_x - P_o}{P_x - P_s}$$

where P_x is the value from crossing, P_s for selfing, P_o for the open-pollination. Similarly a measure of self incompatibility can be determined [45]:

$$\text{Self incompatibility} = \frac{\text{seed set from self-pollination}}{\text{seed set from cross-pollination}}$$

Caution should be employed as self compatibility levels may vary between individuals of the same species and among species [46]. Estimates of the level of cross-pollination can also be determined. The ratio between the number of pollen grains and ovules (the P:O ratio) reflects the breeding system [47]. The out-crossing level of a given species shows a high relationship to the breeding system, the floral size, the temporary separation of anther dehiscence and stigma receptivity, and the spatial relationship of the stigma and the anther [47], although deviations from the P:O ratio standards are now being documented [40].

Concerns about genetic erosion and drift during regeneration

Genetic erosion is the loss of genetic diversity, including the loss of individual genes or particular combinations of genes such as those expressed in landraces. The term “genetic

erosion" is sometimes used in a broad sense, referring to the loss of populations, varieties, or species or more narrowly as the loss of a gene or allele.

Populations are maintained in their natural habitats in unstable equilibrium through the pressures of natural selection. These pressures cannot be emulated during *ex situ* regeneration, and may be replaced by opposing directional pressures, thus leading to accelerated loss of adaptive characteristics. Natural selection depends on the total fitness of individual phenotypes and may operate through: 1) differential survival, and 2) differential fecundity. Both aspects may result in gene loss or a change of gene frequency and a consequent change in mean performance of the population.

The rate of change for any particular trait can be most easily expressed as differential survival [48]. Change will be most rapid where there is genetic variation in highly heritable characters like resistance to disease and pests and some forms of tolerance to environmental stress; least for production characters with low heritability. The intensity of selection and heritability change over environments as a result of genotype by environment (G x E) interactions [49,48,50].

The practical approach during regeneration is to choose environments and cultural practices which reduce the selection pressures. This means ensuring all plants survive and produce seed. However, even when growth and the full reproductive cycle are obtained, natural selection can still operate through changes in the relative reproductive capacities of genotypes in different environments. Although less obvious and dramatic than differential survival, such G x E interactions affecting seed yield are likely to be a major cause of shift in populations during *ex situ* regeneration.

Risk of genetic drift due to sampling errors and genetic shift due to selection may be compounded over regeneration cycles. Frequent multiplication of an accession is another problem, particularly when the sub-sample of seed is too small to be representative of the genetic diversity of the whole accession. Many landraces are heterogeneous mixtures of genotypes, and the problems of genetic drift and shift may be greater. The factors that could cause genetic erosion and drift during regeneration have been summarized [51]. Efficient management of germplasm during regeneration (in the planting, harvesting, threshing, drying and packaging stages) can reduce the risks associated with genetic erosion and drift.

The necessity for, and the problems of, obtaining effective random cross-fertilization and securing equal maternal contributions is related to random drift and the need to know

the appropriate breeding system, pollination method and pollinators. Optimal cultural conditions are essential for the development of high quality seed which is a prerequisite of viability and longevity during storage [52]. In heterogeneous populations the problems of harvesting all plants with uniform ripeness can only be effectively met by harvesting individual plants separately. If the population has to be bulk harvested (as is the case for most genebanks), then a compromise has to be effected, with the emphasis on securing as wide a range of ripe seed as possible [53]. When mature seed is retained by the plant the problem is not great. However, many of the recently domesticated species, particularly forages, and wild and weedy relatives of crop species notoriously shatter seed and this poses special problems in avoiding selective shift.

Variation in flowering time influences phenotypic assortative mating which disrupts the population into extreme types; however, the process is slow unless the heritability is very high [54, 55]. A possible solution is to grow sufficient plants to permit the selection of a large enough number that flower simultaneously [56]. Variation in seed production is more serious and less easily resolved as it involves not only plant production, but also pollinator behaviour. If the desire is to equalize the maternal contribution, the total seed yield of the accession is limited by that of the lowest yielder. Since the importance of the effect on shift and drift increases with the frequency of regeneration, a possible compromise is to save equal maternal quantities for storage and regeneration and bulk the remainder as a multiplication sample for distribution, evaluation and use [57].

Avoiding pollen contamination through effective isolation

Ensuring adequate isolation from pollen exchange among accessions is the single most important step in maintaining genetic integrity and reducing risk during regeneration. Under natural conditions gene flow by pollen can be almost completely counterbalanced by selection against migrant alleles; thus, differentiation may occur in the presence of up to 50% pollen flow, but the selection pressures have to be of a high order – up to 70% to keep the population differentiated in the presence of a pollen flow of 50%~60% [58]. The relationship between the opposing forces of migration and selection are complex, depending on the dominance relationships of individual loci and the inheritance of the characters involved [59,60,61]. In the absence of selection against migrant alleles, such as would be obtained in accession multiplication, even moderate rates of pollen contamination could cause significant alteration [58,62].

There are several ways to achieve adequate isolation, but these depend on the method of pollination (insect or wind) and the reproductive biology of the crop species. In general, isolation is obtained by: 1) spatial or temporal separation; 2) natural or artificial barriers; and 3) hand crossing accompanied by bagging of the inflorescences.

Spatial isolation studies for a number of wind and insect-pollinated species [63,64,65, 65a] all shown significant (>5%) pollen dispersal by both wind and insects can occur over large distances, although this can vary by season, location and species, direction of wind and insect species and their preferences [39]. For example, most wind-dispersed pollen travels much further than animal-dispersed pollen [39]. For seed multiplication of allogamous cultivars, isolation distances in excess of 200m may be a statutory requirement [66,67]. When dealing with a large number of accessions requiring regeneration, isolation by distance is usually not practical. The outstanding feature of both wind and animal pollen travel is its leptokurtic distribution [68,69]. This distribution is influenced by the foraging strategy of the insect pollinators as related to within-plant and between-plant travel [70]. Few studies have investigated gene flow, as opposed to pollen flow, in wind-pollinated plants. In rye grass, *Lolium perenne* over 99% of the genes traveled less than 20m [68] and in beet, *Beta vulgaris*, all genes traveled less than 10m [71]. These values are of an order of magnitude less than the pollen travel observed in these species, and contrast sharply with insect pollinated plants in which gene travel usually exceeds pollen or pollinator travel [39].

Temporal isolation is sometimes practiced on perennial forage grasses [72]. Through cutting, the plots of different accessions are maintained at different stages of development, hence assuring that where crossing is minimal only distant accessions are at the same phenological stage, such as flowering or heading.

Pollen flow can be further interrupted and reduced by intervening barriers of other species, and this has led to the practice of growing isolation plots in standing crops of tall growing species, ie. sunflower, hemp. Several genebanks have exploited this method by establishing seed islands dispersed in crops of winter rye or winter wheat [73] or *Brassica* spp. [74]. Isolation is achieved by 50m separation between the islands and several seed islands may be contained in one field. Each island contains populations of different wind and insect pollinated species, the latter relying on natural pollinators. The assumption is that the pollinating insects remain within a plot, and before approaching another plot they will be attracted by the flowers representing the island plants. Although this method does not ensure the avoidance of any gene flow between accessions, it is relatively simple and cheap and

thus a large number of accessions can be regenerated.

Other methods commonly use artificial (linen, paper bags and other materials) isolations for the period of flowering for wind pollinated species for controlling interaccession pollination [75,76,43]. The bags cover prior to anthesis a single head, 3~4 heads, in a method referred to as cluster bagging, or a whole plot with the intent to encourage cross-pollination and maintain genetic integrity among accessions [77,78]. Supplemental hand-pollination is sometimes required.

The use of screened cages with specially designed hives for honey, leafcutter or bumble bees or flies (hover, blow) for several insect-pollinated crop species is now common [79,43,80,81,82,83]. To save on resources, one accession each of different crop species may be planted under one net or nylon cage. Insect pollinators are released inside the cage at flowering time to enhance pollination within each accession and thus increase yield. However, problems may occur when more than one accession is grown under a cage, and in come cases supplementary hand-pollination may be necessary, eg. wild species of tomato and eggplant. Also insect pollinators may show preference for one species. The USDA tested the ability of cages to restrict gene (pollen) flow [84]; compared seed produced by various races of honey bees [85]; and documented improvements in regeneration quality and quantity - for sunflower and carrot. Purpose-built pollen proof glass or polyethylene houses are effective, but expensive to operate and maintain [88]. An example can be found at the Institute of Grassland and Environmental Research, Wales which uses a rigorous regeneration scheme for all accessions (forage grasses and clovers) using elaborate crossing houses [89].

The use of isolation cages poses several problems. Aside from being expensive and laborious to construct, the shading that results affects plant growth and may distort characterization data, and pollinator foraging behaviour [90.43]. The environment inside a cage may be favourable for pest insects (aphids, mites); severe infestations may be frequent and often difficult to control even with repeated use of pesticides. Their use may result in negative pesticide-pollinator interactions and the need to reintroduce adequate numbers of pollinators. On the positive side, cages may result in the exclusion of pest, such as protecting Cucumis from beetle-transmitted bacterial wilt [91] or other insects that damage plants and/or fruits, which cause reduced seed yield as well as reduced seed vigour.

The ultimate effective isolation is controlled pollination by hand. During the early stages of seed regeneration hand-pollination can produce sufficient seeds, but with

expanded programs for breeding, testing and the handling of large collections with numerous accessions of each species and the need for effective population size to maintain integrity this becomes too laborious and time consuming. Furthermore, natural pollinators are more efficient and effective in producing quality germplasm than by hand.

Pollinator choice and management

By definition cross-pollination simply means the transfer of pollen of one flower onto the stigma of another by a vector. Efforts have focused on the use of commonly available pollinators for specific crop species with significant success using the honey bee, *Apis mellifera*, and the alfalfa leafcutter bee, *Megachile rotundata*, and lesser success with other bee species such as the mason bees, *Osmia* spp. and bumble bees, *Bombus* spp. and some fly species such as syrphids, *Eristalis tenax* and blowflies, *Phormia terranovae*.

The challenges facing genebank curators in using insect pollinators to regenerate cross-pollinated species are numerous and need addressing through research. They include:

1. identify the optimum (most efficient and effective, but not necessarily the most abundant) pollinator for each crop species; this is compounded as many accessions are being regenerated in regions far from their origin with the optimum pollinator lacking at the new site; particularly troublesome are plant species, frequently native ones, which are oligophilic;
2. identify the 2nd and 3rd pollinator species and their relative value;
3. distinguish between visitors to flowers and actual pollinators, i.e. those that collect, transport and deposit compatible pollen;
4. develop a standard methodology for determining optimum pollinators;
5. number of pollinators needed to pollinate flowers in small plots, cages or greenhouses; integration of bee behaviour, plant yield and agronomy into a pollination model to predict the probability of an individual flower being pollinated under various pollinator and flower densities; the number will be different from large-scale field production; need to account for small plot size, pollinator drift, and nearby plant competition;
6. management techniques (equipment and costs) for the different pollinator species varies and needs to be learned or developed; recent introductions of non-Apis pollinators to new regions have met with varying success [92] ;

7. many genebanks have well trained staff in plant sciences to manage plant regeneration, but not in entomology to manage pollinators; also specialized facilities and training may be needed; and
8. introducing oligolectic pollinators to new regions may increase the risk (new diseases, pests) to already existing pollinator species and disturb existing native plant communities.

Characterization & evaluation

Characterization and evaluation are the functions where value is added to each accession. To avoid confusion when building capacity and in general use characterization means analyzing characters which are highly heritable, that can be seen easily by the eye (except molecular characters) and are expressed in all environments (color and morphology). Evaluation referred to analyzing characters not as easily observed visually, such as pathogen or pest resistance or quality characters (oil or protein content, fiber content, fatty acid profile).

Curators must be aware of germplasm users' (especially breeders) needs for information required to register new cultivars and focus on acquiring this information. Agronomic and horticultural data are high priorities, but quality traits and disease-resistance data are also highly desired. Curators must also be aware of new diseases and respond quickly with evaluations for host-plant resistance. Some countries may not be devoting enough time/resources on evaluating germplasm for disease resistance or product quality, sometimes because a standard set of differential lines (isolates) for certain pathogens are lacking. The development of core (or sub-core) subsets is needed for more crop species. Core subsets provide one rational mechanism for managing the genetic diversity within a collection.

Considering its global importance, it is striking that climate change is rarely mentioned in rationale or objectives for most gene banks. Curators and managers should assume a proactive role in preparing for this so as to minimize future crop production risk. Relevant germplasm should be acquired, new descriptor criteria (eg. resistance to drought, salinity, identification of stress tolerant genes) and techniques developed, and germplasm evaluated for those factors.

Examples of characterization & evaluation research to reduce risk

Analyses of phenotypic variation for 10,050 accessions of hexaploid cultivated oat were completed. Eight environmentally stable morphological characters differentiated the germplasm into 18 character states. Comparisons of oat diversity from different countries, among the major infraspecific groups and among Canadian oat cultivars registered between 1886 and 2002 were possible. The number of accessions in each morphological group was unevenly distributed with the 13 most frequent morphological groups representing 90% of the accessions. The most frequent morphological groups were identical to the most frequent types of Canadian oat cultivars. The greatest richness of diversity was found in oat from countries with temperate climates and intensive oat breeding programmes. The oat accessions comprised 8754 accessions of common hulled oat, 183 accessions of hull-less oat and 1168 accessions of red oat. For red oat *(A. sativa* (=*A. byzantina* C. Koch)), West Asia was richest in diversity. The USA could be considered a secondary centre of diversity for red oat and Canada a secondary centre of diversity for hull-less oat [93].

Modern molecular tools, such as AFLP (amplified fragment length polymorphism), SSR (simple sequence repeat) and DNA sequencing, are being used to characterize genetic diversity in plant and animal populations. Effective genetic analyses including Bayesian and stochastic modeling approaches are applied to partition genetic diversity within and among populations, whereas phylogenetic reconstruction methods retrace and illustrate molecular ancestry and relationships among the plant or animal groups of interest. Various diversity functions (Weitzman and Marker Estimated Kinship) help set priorities in plant and animal germplasm.

Germplasm characterization with molecular genetic markers has increased substantially. Although genetic marker information provides new insights into intrinsic genetic variability, the analyses and application of genetic markers involve many challenges. National workshops to discuss these challenges and of integrating molecular data into practical gene bank research would be valuable. They could address the optimal types of marker, strengths/weaknesses for specific purposes, and the appropriate number of markers. For some crops, it may be more efficient to involve industry or university partners or to farm-out genetic marker analyses rather than to develop the expertise “in-house.” This approach may increase interaction, take advantage of their expertise, increase utilization of collections and enhance capacity building within and among nations.

Diversity analyses based on molecular and agrobotanic studies have been conducted on wild and cultivated germplasm of flax [94,95,96] and oat [97,98,99] and several oilseed crucifer species [100]. These studies have resulted in more efficient germplasm conservation, and useful germplasm has been identified and integrated into breeding programs. Molecular analyses of the genetic diversity of wheat [101,102], soybean [103] and several native Canadian grass species [104,105,106,107,108,109] have also justified the need for germplasm conservation.

AFLP analysis of 163 accessions of 25 *Avena* species with diverse geographic origins was completed. This analysis revealed 59.5% of the total AFLP variation resided among 25 oat species, 45.9% among six assessed sections of the genus, 36.1% among three existing ploidy levels, and 50.8% among eight defined genome types. All the species were clustered together according to their ploidy levels. The C genome diploids appeared to be the most distinct, followed by the Ac genome diploid *A. canariensis*. Analysis of AFLP similarity suggested that the AC genome tetraploid *A. maroccana* was likely derived from the Cp genome diploid *A. eriantha* and the As genome diploid *A. wiestii*, and might be the progenitor of the ACD genome hexaploids. These AFLP patterns are significant for our understanding of the evolutionary pathways of *Avena* species and genomes, for establishing reference sets of exotic oat germplasm, and for exploring new exotic sources of genes for oat improvement [110].

SSR analysis of 169 potato accessions representing diverse geographic origins was completed. This analysis showed 2.4% of the total SSR variation resided among accessions of different countries, 0.8% between Canadian and exotic potato accessions, 3% among Canadian accessions of four breeding periods defined, and 0.8% between Canadian heritage and non-heritage cultivars. These findings demonstrate the narrow genetic basis of potato germplasm and justify the need for broadening the improved potato gene pool. In spite of this, the most genetically distinct potato germplasm was identified for potato improvement [111].

The Microbial collection has contributed to research on several plant diseases and mycotoxins and provided information to national and international clients. Initial efforts to develop broad-spectrum diagnostic kits to detect *Carlaviruses* have been successful and prototype RT-PCR primer sets were used to detect and sequence two new highly divergent strains of Blueberry scorch virus in BC.

Evaluation of disease resistance in chickpea, canola and lentil relies on fungal isolates representing pathotypes, inoculation in growth chambers and verification with natural

infection in a cold-frame greenhouse. The genetics of resistance inheritance will requires the segregation of resistance in F_1, F_2 and backcross generations, populations of doubled haploid lines and/or recombinant inbred lines. Mapping of sclerotinia resistance involves microsatellite markers, and mapping of ascochyta resistance will use single nucleotide polymorphism.

Indoor disease screening methods were developed for lentil, canola, bean, sunflower, soybean and chickpea and a 4000 sq ft cold-frame greenhouse was built for screening under natural infection; QTL's conferring sclerotinia resistance in canola were identified, and data on 1500 lentil accessions of which 35 were resistant to anthracnose were placed in GRIN-CA. Bioactive substances in diverse germplasm of pea and lentil have been isolated and identified.

Evaluation of canola (*B. napus*) germplasm for resistance to the fungal pathogen *Sclerotinia sclerotiorum* the cause of stem rot: QTLs conferring resistance to sclerotinia stem rot in the Chinese cultivar Zhong You 821 (ZY821) were mapped in 6 DH and 3 RIL populations. So far, QTLs have been identified on LG 1, 3 and 19. *B. napus* germplasm from PGRC, Dr. Rakow (AAFC, SRC), and the Nordic and Czech gene banks were screened for stem rot resistance; up to now, 10 new lines, obtained from Kashmir and Pakistan, have high levels of stem rot resistance. New methods for production of ascospores under controlled conditions and inoculation of canola stems, with ascospores on petals as nutrient source, were developed. Two sclerotinia nurseries were used to screen selected resistant and susceptible DH lines and germplasm under field conditions; however, inoculation of the soil with sclerotinia (survival structures) created variable inoculum pressure confounding the disease rating; consequently, inoculation of individual plants with ascospores on petals is now used. A micro array study of genes differentially up or down regulated in infected versus un-infected stem tissue, and susceptible versus resistant tissue were conducted and approximately 50 potential defence genes were identified.

Bioactive compound identification and seed oil evaluation uses various chromatographic techniques (medium pressure liquid, flash, thin-layer and gas) as well as electrospray – ionization, and matrix-assisted laser desorption/ionization mass spectrometry, and high-resolution NMR spectroscopy; concentrations will be assessed using HPLC with evaporative light scattering and photodiode array detectors.

Six chickpea seed samples supplied by Crop Development Centre, University of Saskatchewan were ground and flour samples (1-gram scale) extracted at ambient

temperature with 80% methanol. The crude extracts, treated briefly for 7 days with ordinary fluorescent light, were cleaned with Diaion HP-20 beads before soyasaponin analysis by HPLC. Defatted flours (100g) of the yellow field pea, green field pea, lentil, dry bean and soybean were processed in methanol with brief and 3 days of fluorescent light exposure in a chamber. The HP-20 methanol fractions from these extracts were isolated and examined by HPLC and electrospray LC/MS. The best source of dehydrosoyasaponin I was Amit chickpea followed by soybean and yellow field pea. Data was tabulated and submitted for inclusion into a PCT application.

Raw Amit chickpea flour (100 g scale), a controlled environment chamber maintained at 21C and equipped with 6x40 Watt fluorescent lights of the Cool White type (intensity of about 2250 lux at the centre of the chamber) proved to be an excellent visible light source for the generation of dehydrosoyasaponin I from soyasaponin VI. The utility of this chamber was demonstrated in two experiments with 80% methanol (48 hour light exposure) and two experiments with 60% ethanol (72 hour light exposure) as extraction solvent. Resulting Diaion HP-20 methanol (and ethanol) fractions enriched in dehydrosoyasaponin I (and soyasaponin I) were studied in detail by TLC, HPLC and electrospray LC/MS. Using these fractions (50 mg/injection), it was demonstrated that dehydrosoyasaponin I could be isolated in milligram quantities by medium pressure liquid chromatography with a custom-prepared reverse phase column of styrene/divinyl benzene (Source™ 15 RPC) and a gradient of ammonium hydroxide plus acetonitrile. Data was tabulated and submitted for inclusion into the PCT application.

Research on animals uses standard procedures for collection of germplasm from livestock and poultry breeds, extraction of proteins, Western blotting, immunolocalization, SDS-PAGE, 2-D electrophoresis, RT-PCR, and *in situ* hybridization. *In vitro* production of swine embryos depends on three techniques: *in vitro* maturation of oocytes, *in vitro* fertilization, and *in vitro* culture of embryos. Modifications to conditions and media are implemented to optimize embryo production. To transfer pig embryos, non-surgical methods are being developed. Modification of cryo-media and cryopreservation protocols are being developed to preserve bull, boar and rooster semen. Vitrification techniques are being used to preserve oocytes and embryos from different species.

Documentation

A computerized database management system (GRIN-CA) to handles the massive amount of data collected by PGRC, the Clonal Gene bank, the Nodes and the Animal Genetic Resources Program. The database software was acquired from the USDA, Database Management Unit, Beltsville, MD which means that all plant genetic resources in North America use the same database system. A new database structure is being developed for the animal program in cooperation with the Animal Genetic Resources Unit, Ft Collins, CO and EMBRAPA, Brazil. Combined, these systems with common programming language increase the programming capability and reduce the risk to loss data. The computer hardware/operating system used in Canada is a Sun/Solaris with an Oracle user interfaces supplemented by MVC web technology and traditional HTML. Timely back up of program language software and all data is essential to reduce risk of loss. Periodic placement of data at another location is highly desired.

Increased emphasis is being placed on digital imaging, especially when images of different organs (seed, flower, roots) are being captured so as to provide validity to descriptor characters. The next step will be to apply new technologies to numerically scan the images and generate numerical data as well. A major effort is still required to incorporate genetic marker data into GRIN-CA and this will be challenging.

Capacity building

Capacity building in Canada takes many forms. A senior undergraduate course in plant genetic conservation and utilization is offered by genetic resource staff in the Plant Science Department, University of Saskatchewan. Animal genetic resource staffs offer several lectures in animal genetic resource conservation within the Animal/Poultry Science Department and the Western Veterinary College, University of Saskatchewan. The ultimate aim here is to develop a formal graduate degree granting program in animal genetic resource conservation. Training of a number of undergraduate, graduate and post doctoral students occurs as do numerous shorter term visits by international colleagues. Numerous tours of facilities and collections are given to scientific and producer societies or organizations, parliamentarians, visiting delegations and school groups. Written productivity takes the form of peer reviewed scientific publications, posters, technical reports, abstracts, book reviews, book chapters and numerous presentations at scientific and producer meetings.

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