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The value of national fruit gene banks

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In recent years, several national fruit gene banks have been established in the temperate zone as a response to decreased diversity in the assortments used in pomological research and production. Due to limited funding, one of the important challenges facing these gene banks is to select nationally representative material and to create cost-effective programs of conservation. In addition, we argue that gene banks can substantially increase the value of their collections by systematic evaluation of the material, using traditional and modern methods. If the accessions are carefully described and their traits well documented, their usage in commercial production, product development and research is facilitated. For example, gene banks can assist future breeding programs through helping to create saturated gene linkage maps for important fruit species and by adding descriptions of non-horticultural traits such as the content of phytochemical substances. In this review article, we discuss fruit gene bank management and highlight important areas of research for curators. Black currant, sea buckthorn and blue honey suckle are used to illustrate the importance of phytochemical mapping.

Key words: fruit germplasm, genebank management, phytochemical diversity

INTRODUCTION

In the last decades, the number of varieties grown by commercial fruit growers and nurseries has diminished. As a response to this depletion, an increasing interest in national fruit gene banks can be noted. The overall aim of national gene banks is to safeguard the pomological heritage and make sure that unique and valuable germplasm is not discarded. However, for practical and economic reasons it is impossible to preserve all the available material. Thus, successful gene banks must focus on selecting and conserving representative material carefully. In this aspect, gene banks are similar to financial institutions. The deposited capital should be managed in such a way that its value increases to the benefit of future generations. The management should be secure and cost-effective.

Even though gene banks are of recent date, man started to collect plant genetic resources long ago. Probably the first fruit collections were established on farms where spontaneous selections of promising genotypes led to the introduction of local varieties. Systematic conservation of plants began at botanical gardens, and in 1894 the first well documented gene bank, N. I. Vavilov All-Russian Institute of Plant Industry (VIR), was established. Already from the start, scientists at VIR collected a diversity of genetic resources from all over the world. Today VIR has a global collection of fruit, berries and grapes, which contains approximately 24 500 accessions. The material is maintained in a network of 12 research stations in different parts of Russia [4]. In the 1990s, following independence, several former Soviet states, including Lithuania, have established their own national fruit gene banks.

In the United States, the development of gene banks has been different to that in Russia. Up until 1980 fruit and nut germplasm was maintained at state universities and experimental stations as working collections of individual scientists and breeders [14]. Consequently, the survival of the material depended on the activities of individual researchers. Thus, irreplaceable material could easily be lost due to retirement and shortage of funds.

To remedy this situation, the U. S. Department of Agriculture, along with state experimental stations, began the establishment of a national germplasm system in 1980. Today, the system comprises eight gene banks, so-called repositories, devoted to long-term conservation of fruit and nut crops and their wild relatives. Altogether the repositories maintain some 30 000 accessions representing 1600 species. Each repository is responsible for holding globally diverse collections of assigned crops. Fruits from temperate regions are preserved at the repositories at Brownwood (Texas), Corvallis (Oregon), Davis (California) and Geneva (New York). Pears and berry crops are assigned to Corvallis, while apples and cherries are maintained in Geneva.

During the 1980s, the situation in Western Europe and Scandinavia was similar to that of the U. S. The governmental funding for breeding, pomological research, including evaluation of fruit cultivars in comparative trials, was in decline and thus the base for applied research, i. e. the fruit collections, was endangered. This trend drew attention to the importance of national gene banks and eventually led to the establishment of several European repositories. Gene banks do not only preserve our pomological heritage for future generations; they are also important means for education and creating awareness of biodiversity among students and the general public. In addition, they provide raw material for basic plant genetic research and can be used to develop new and better products to meet the needs of today and tomorrow. To improve the safety of conservation and increase the value and usages of the collections, it is important for gene banks to integrate genetic mapping and phytochemical characterizing in germplasm management.

GERMPLASM MANAGEMENT AND GENETIC CHARACTERIZATION

The mission of national genebanks includes collection, identification, preservation, evaluation, documentation and distribution of plant genetic resources. Traditionally, fruit genetic resources are preserved in field collections with back-up plants for each accession. Some crops, such as stone fruits, raspberries and currants, are subjected to insect-borne viruses and must be preserved in screenhouses to prevent infections. Maintaining more than one plant of each accession increases the security of the collection, but also the cost related to preservation. Thus, decreasing financial support for long-term storage can cause a danger of germplasm erosion.

The accessions preserved in the national collections are commonly described using internationally acknowledged morphological, phenological and horticultural descriptors such as those recommended by the International Union for Protection of New Varieties of Plants (UPOV). It is important to describe virus-free specimens, so that virus symptoms are not described instead of plant characteristics. Description work is crucial because only well characterised genetic resources can be used efficiently for breeding and pomological research. Another objective for gene banks is to conduct appropriate research with the aim to develop and introduce improved methods for germplasm conservation and genetic characterization, i. e. to enhance future preservation and utilization [20].

An example of gene bank management is found in the U. S. where the fruit gene banks in Corvallis, Davis and Geneva cooperate within the framework of the national research program for Plant Genetic Resources, Genomic and Genetic Improvement [2]. The main focus of research within this program is to combine traditional field evaluations with biochemical and molecular characterization using PRC-based markers such as microsatellites, AFLPs and cpDNA sequences. Characterization using molecular genetic markers is important for determining the levels of genetic diversity and identifying genetic gaps and duplicates in the collections, thus facilitating their effective management.

Another example of research-oriented gene bank management is found in the U. K. where genetic fingerprinting for all 2300 apples and some 250 pears in the national fruit collection is now carried out by East Malling Research (EMR) under a three-year contract [1]. Additional 295 varieties of pears and 200 varieties of cherries have already been fingerprinted. DNA-markers are used to obtain individual profiles for each accession in the collection and can, along with observations in the field, assist curators to resolve uncertainties and verify the uniqueness of the material. At EMR, the collection is genetically described using an internationally established set of reference microsatellite markers. Thus, it will be possible to compare the varieties in the U. K.'s national collection with those in other countries.

In addition, there have been several other national and international research projects with the objective to develop DNA markers to describe the germplasm of fruit species within the *Rosacea* family. According to Dirlewanger et al. [5], a *Prunus* reference map with 562 markers is available and a further set of 13 maps has been constructed with a subset of the markers, which allows genome comparison among seven *Prunus* diploid (x = 8) species. In all of these markers, colinearity was the rule. Furthermore, following the preliminary results, a comparison between apple and *Prunus* maps suggests a high level of syntony between these two genera. It is also reported that markers linked to major genes, i. e. genes responsible for the expression of important traits including disease and pest resistance, fruit quality and self-incompatibility, have been developed for apple and *Prunus* and are already considered a useful tool for marker-assisted selection in current breeding programs. In addition, similar work of a quantitative character using linkage maps and candidate genes is in progress. The authors conclude that genomic information such as the *Prunus* physical map, large EST collections in both *Prunus* and *Malus,* and the establishment of the map position of high numbers of ESTs is needed to obtain better knowledge of the large *Rosaceae* genome.

The apple genetic linkage maps so far developed have been composed of isozymes, random amplified fragment polymorphisms (RFLPs) and simple sequence repeats (SSRs). However, the maps need to be better saturated with more robust markers to allow transfer to additional apple cultivars and populations. Thus, a genome-wide framework for a high-resolution physical map of the apple genome by BAC fingerprinting is under development at the University of Illinois, Urbana, U. S. [7].

IN VITRO AND CRYOPRESERVATION

The future management systems of gene banks for vegetatively propagated crops will have to meet several challenges – not only the transition from the acquisition phase to the evaluation phase, but also a more efficient conservation and utilization of the plant material. Thus, the management activities of large national gene banks are often supported by research programs that deal with pathogen testing and therapy, tissue culture, *in vitro* and cryogenic storage.

Ideally, gene banks should preserve and distribute plant material free from virus diseases. At the clonal germplasm repository in Corvallis, U. S., the accessions are tested for viruses by inoculating a sensitive indicator plant and by using Enzyme Linked Immunosorbent Assay (ELISA) and Reverse Transcription Polymerase Chain Reaction (RT- PCR) [3]. Plants which are infected with viruses are subjected to heat therapy for several weeks at temperatures around 38 °C. After the therapy meristems less than 1 mm in length are collected from shoots produced during heat therapy. From these meristems, new plants are regenerated and tested to see if the virus was indeed eliminated. The methods for virus detection and elimination presented above are also used in certification programs for production of virus-free elite stocks from fruit cultivars. Following virus elimination, the material is checked to ensure that no genetic changes have occurred from the therapy.

Maintaining multiple plants of each accession in field collections as a backup increases the safety, but it is costly and reduces the space available for additional accessions. Some genebanks use *in vitro* culture as a back-up, while others use cryogenic preservation of either apical meristems or dormant buds. The *in vitro* culture at the repository in Corvallis serves several purposes [16]. Firstly, it is a space-saving back-up for the field collection. Secondly, the *in vitro* collection stores plants in a sterile environment free from viruses and most diseases. Another advantage of *in vitro* plants is that they can readily be distributed. In Corvallis, approximately 1500 accessions are stored at 4 °C. It is important that *in vitro* collections are supervised properly. Data on the storage life of individual accessions should be noted and the accessions should be subjected to genetic stability tests. The storage life of individual plants is typically 1.5 to 4 years depending on species.

Long-term storage of clonal germplasm can also be achieved through freezing meristems and pollen in liquid nitrogen. The curators at Corvallis emphasise that the meristems must be pre-treated with a cryoprotectant (antifreeze) and frozen in a very controlled manner before being plunged into liquid nitrogen at −196 °C [16]. The pear cultivars and clones preserved at Corvallis are backed-up cryogenically at the National Seed Storage Laboratory in Ft. Collins, Colorado. The technology is continuously evaluated in research studying pre-treatment techniques, cryoprotectants, freezing protocols, recovery stages and genetic stability of stored meristems.

At the repository in Geneva, U. S., two clones of each accession of apples are maintained in the field gene bank, a procedure which tends to be expensive due to the large number of accessions maintained [6]. Calculations show that it costs 75 to 100 US dollars per year to keep a single tree in the field collection, but only 1 dollar to preserve it cryogenically. This fact, along with the collections vulnerability to insects, pests, diseases and natural disasters, has led curators to suggest that it is no longer advisable and cost-effective to maintain the entire collection in field. During the last 15 years, 750 accessions of apples kept at Geneva have been cryopreserved as back-up at the National Seed Storage Lab. in Ft. Collins along with an additional 250 accessions in Geneva. In contrast to pears, bud segments are placed in cryo instead of meristems. Tests to recover the frozen buds have been promising. Successful recovering by grafting was obtained from over 90% of 600 accessions tested. These results indicate that cryopreservation may be a safe and cost-effective way to enhance the management of fruit germplasm.

PHYTOCHEMICAL MAPPING OF HEALTH-PROMOTING COMPOUNDS

Describing the content of phytochemical substances in gene bank accessions may enhance the value and usages of the preserved material. In the following, the benefits of phytochemical mapping is discussed in general as well as for three specific species (black currant, sea buckthorn and blue honey suckle).

Today's consumers are focused on quality and purity as well as environmentally safe production, a phenomenon that has created new interest within the food industry, i. e. functional foods. Typically, these products are natural elements of the diet, can be consumed without apparent risk of an overdose and have a documented desirable effect on important physiological functions. For example, they can strengthen the immune defence and prevent illnesses. It is common that these new food products are based on vegetable raw material or include specific substances of plant origin.

Several fruit, berry and nut species have qualities that are attractive for producing functional foods. Additionally, aroma, flavouring, thickeners, antioxidants, preservatives, vitamins and minerals are often made from plant extracts which give the final food product a natural touch. Small fruit have long been regarded as having considerable health benefits, particularly due to their content of antioxidants which limit cellular oxidation reactions. These benefits have stimulated research to investigate the antioxidant capacity of berries. The antioxidant character is strongly affected by the type of berry (species and varieties within species), but it can also be affected by cultivation conditions. The antioxidant capacities of small fruits are based on their content of antioxidant phytochemicals such as phenols, anthocyanins, flavonoids, catechins, carotenoids and vitamin C [17].

Given this new focus and the fact that the phytochemical properties of cultivars no longer in commercial production are not documented, it is important to conduct phytochemical mapping of gene bank collections. Further, until now little attention has been paid to studying the processing characters of fruit and berries. For instance, raw materials from varieties with a high content of bioactive compounds need to be processed carefully to preserve the valuable health-promoting properties in the final product.

Phytochemical mapping at gene banks gives opportunities to select varieties with a high content of bioactive compounds. In a second stage, experimental fruit and berry processing centres should work with development and pilot production of functional food and natural food components. Through pooling the competences of natural product chemistry and pilot processing facilities with ongoing germplasm conservation, genetic research and breeding programs, it will be possible to extend the use of selected old cultivars as well as develop new cultivars aiming at health promoting products in cooperation with the food industry.

Black currant *Ribes nigrum* L.

Black currant belongs to the genus *Ribes* and the *Eucoreosma* section of the subgenus *Coreosma*. The main geographical centres of diversity for black currants are northern Europe, Scandinavia and Russia [8]. Black currant is an important raw material in the food industry due to the high content of ascorbic acid (127–198 mg/100 g) and anthocyanins (308–912 mg/100 g) in mature berries. Seed oil from black currant is known to be one of the richest natural sources of gamma-linolenic acid, with values of up to 20% of this acid in the seed oil. These concentrations are sufficient for most but not all applications of the oil [18]. Nilsson and Trajkovski [12] investigated the distribution of colour pigments in species and hybrids of the genus *Ribes* L. The results have shown that the main anthocyanins of *Ribes nigrum* L. are cyanidin-3-rutinoside and delphinidin-3-rutinoside, followed by cyanidin-3-glucoside and delphinidin-3-glucoside. A characteristic of black currant leaves is the high content of flavonols and phenolic acids. The main flavonol compounds are quercetin-3-monogalactoside, quercetin-3-monoglucoside, quercetin-3-rhamnoglucoside, kaempferol-3-monogalactoside, kaempferol-3-monoglucoside and kaempferol-3-rhamnoglucoside. In addition, chlorogenic, isochlorogenic, neochlorogenic and caffeic acids are the major phenolic acids in developed leaves. Young shoots and leaves mainly contain flavonol compounds, phenolic acids and ascorbic acid [19]. It is well known that the leaves and buds of black currants and other *Ribes* species have a strong odour due to high contents of volatile oils. The oils are used as a component in some French perfumes and to reinforce or modify black currant flavour. Black currant bud oil is very complex and contains numerous substances; 123 of them have been detected, of with 66 have been identified by Kerslake and Menary [10]. The main substances are monoterpene hydrocarbons (about 80%) and sesquiterpene hydrocarbons (about 12%). The powerful and characteristic odour is attributed to 4-methoxy-2-methyl-2-mercapto-butane.

Sea buckthorn, *Hippophae rhamnoides* L.

The genus *Hippophae* L. belongs to the family *Elaegnaceae* and is widely distributed on the Eurasian continent, and the species *H. rhamnoides* L. is the most widespread and economically important species in the genus. Sea buckthorn is a dioecious plant and forms a shrub or a small tree with nitrogen fixing root modules tolerating floods, periodical droughts and hard winters, binds soil effectively and is thus an important natural or cultivated species in eroded areas. The berries have been known and used by humans for centuries both in Asia and in Europe. Russia and China are leading countries in domestication, breeding, cultivation and introduction of products on the market. The berries unsuitability for mechanical harvest is the main problem for developing an efficient production system.

Besides having a very exclusive aroma, sea buckthorn berries are rich in vitamins, especially ascorbic acid with concentrations between 28–201 mg/100 g among individual genotypes in a population of *H. rhamnoides* subsp. *Rhamnoides* [21]. Higher levels and larger variation of ascorbic acid (1120–1440 mg/100 g) was noted among the genotypes of the Chinese *H. rhamnoides* subsp. *sinensis* [11]. Another characteristic of the berries is the high content of oil in the pulp and seeds. The oil content in pulp and seeds is 6.8–7.5% and 10.2–12.0%, respectively. Carotene content in the pulp oil is 54.0–102.6 mg/100 g, which is more than in other fruits or vegetables. The vitamin E content in pulp and seed oil is 40.1–62.8 mg/100 g and 65.7–104.1 mg/100 g, respectively. The pulp oil contains 68.8% of unsaturated acids; nexadecenoic acid (35.0%), oleic acid (25.2%) and linoleic acid (45%) are dominant, and the seed oil contains 88.9% of unsaturated acids, among which oleic acid (23%), linoleic acid (37.0%) and linolenic acid (27.6%) are dominant.

The total flavonoid content in the berries ranges from 0.2–0.7%, and more than 10 flavonoid compounds have been identified [15]. In addition to antioxidant vitamins, another antioxidative capacity, superoxide dismutase, is high [9]. The contents of all these components vary greatly according to the species and subspecies within the genus *Hippophae*. Growth conditions and stage of ripeness are the other factors affecting the amount of antioxidantia.

Blue honeysuckle, *Lonicera caerulea* L.

The genus *Lonicera* L. belongs to the family *Caprifoliaceae*. The species *Lonicera caerulea* is distributed throughout the temperate northern hemisphere. There are nine varieties of edible honeysuckle, treated as subspecies. Domestication and breeding of *Lonicera caerulea* var. *altaica* and *Lonicera caerulea* var. *kamtschatica* was performed by Maria Plekhanova and co-workers at VIR [13]. Good tasting, sour-sweet berries with a nice aroma are characteristic only of *Lonicera caerulea* var. *kamtschatica* growing in the Asian part of Russia and eastern Siberia. It is from seedlings of this particular subspecies that the first varieties of blue honeysuckle have been selected. The berries ripen almost at the same time as strawberries when no other berries are available for processing. The berries weigh 1.5–2 g and have a length of 3–4 cm. Ripe berries can easily be harvested both manually and with a mechanical harvester similar to the machines used for black currants.

The berries have a high content of anthocyanins (1400 mg/100 g), phenolic carboxylic acids (160 mg/100 g), flavonoids (140 mg/100 g) and ascorbic acid (40–170 mg/100 g). The major anthocyanins are cyaniding-3-rutinoside and cyaniding-3-glucoside. Berries are consumed fresh or used for production of jam, juice, baby food fruit mixtures and beverages as well as for obtaining natural food colourant with a dark red colour. The domestication and breeding program with blue honeysuckle is also performed by Maxine Thompson at the National Clonal Germplasm Repository, Corvallis, U. S. In this program, progenies from the subspecies *Lonicera caerulea* var. *emphyllocalyx* derived from seeds collected at different localities in Hokkaido, Japan are evaluated.

CONCLUSIONS

Following in the footsteps of pioneering gene banks such as VIR, several new national repositories for fruit and berries have been established in recent years. The primary goal of these gene banks is to prevent unique and valuable genetic resources from being lost. In this paper, we argue that the value of these collections can be enhanced through systematic evaluation of horticultural, phytochemical and genetical properties. In most fruit crops, there is a substantial genetic variation of commercially important traits such as growth habit, fruit colour, yield, taste, nutrients and antioxidantia. Through the description of those traits, breeders and product developers can be helped to select appropriate plant material for specific purposes. Breeders, as well as curators, can be further assisted in their work by gene mapping with robust markers. Up until now, only a small portion of the genes hidden in our fruit and berries have been identified and linked to important traits. The better the material is described the easier it will be to identify interesting properties and make sure that the whole spectra of variation is preserved. Furthermore, we argue that gene banks ought to conduct research on preservation methods with the aim to increase safety and cost effectiveness. In summary, gene banks represent a value in and of themselves. However, this value can be substantially increased, if the preserved material is well documented. Only in this way gene banks will give a proper return to society for its investments.

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NACIONALINIŲ SODO AUGALŲ GENŲ BANKŲ REIKŠMĖ

Santrauka

Vidutinio klimato zonoje dėl mažėjančios genetinės įvairovės pomologiniuose tyrimuose ir versliniuose soduose pastaraisiais metais įkurti keli nacionaliniai vaisinių augalų genų bankai. Šie bankai kol kas prastai finansuojami, todėl jų pagrindinis tikslas yra kaupti vaisinių augalų rūšis, vietines veisles ir kurti kuo pigesnius bei efektyvesnius jų saugojimo metodus. Teigiame, kad sistemiškai tiriant turimą medžiagą tradiciniais ir moderniais tyrimo metodais šių genų bankų kolekcijų vertė padidėja. Tinkamai aprašius turimą medžiagą ir detaliai įvertinus tam tikrus požymius labai palengvinamas jų panaudojimas vaisių komercinėje gamyboje, kuriant ir tiriant produktus. Pavyzdžiui, genų bankai gali padėti selekcininkams sudarant bei tobulinant genolapius ir aprašant šių augalų neagronominius požymius, tokius kaip vaisių cheminė sudėtis.

Šiame straipsnyje aptariamas genų bankų valdymas ir pabrėžiamos svarbiausios tyrimų sritys. Fotocheminio apibūdinimo reikšmei parodyti panaudoti juodieji serbentai, šaltalankis ir melsvauogis sausmedis.

Raktažodžiai: augalų genetiniai ištekliai, genų banko valdymas, fitocheminė įvairovė

Виктор Трайковски, Ингер Гялмарссон

ЗНАЧЕНИЕ ГЕННЫХ БАНКОВ ПЛОДОВЫХ РАСТЕНИЙ

Резюме

В связи со снижением генетического разнообразия ассортимента, используемого в помологических исследованиях и в промышленном садоводстве, в странах средней полосы в последние несколько лет были созданы национальные генетические банки плодовых растений. Из-за недостаточного финансирования одна из основных задач банков – отбор национального материала плодовых растений и создание эффективных программ для его сохранения. Мы утверждаем, что генетические банки могут существенно повысить значение своих коллекций при их систематическом изучении традиционными и новыми методами. При тщательном описании признаков образцов и хорошей документации увеличиваются возможности использовать их для коммерческого производства плодов, создания и изучения продуктов переработки. Например, генетические банки могут помочь селекционерам создавать генетические карты важнейших видов плодовых растений и описывать такие признаки, как количество и состав фитохимических субстанций. В настоящей работе обсуждается управление генетическими банками, намечаются основные направления исследований. На примере черной смородины, облепихи и синей жимолости показано значение фитохимического изучения растений.

Ключевые слова: генетические ресурсы, управление генетическими банками, разнообразие фитохимического состава