Mitteilungen Klosterneuburg 60 (2010): 435-441

Conservation, description and sustainable use of temperate fruit biodiversity

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Biodiversity in temperate fruit species has considerably eroded in recent decades in many fruit producing areas of the world. But based on the Convention on Biological Biodiversity (CBD) and the Global Plan of Action several countries have strengthened efforts for the conservation and sustainable use of plant genetic resources. In Switzerland considerable progress has been achieved in detecting, conserving and characterizing fruit genetic resources. Guidelines for the phenotypic description of fruit genetic resources were developed and applied. Moreover, molecular markers were applied in order to detect duplicates allowing to rationalise genebank collections.
Keywords: biodiversity, fruit genetic resources, fire blight, fruit quality

Erhaltung, Beschreibung und nachhaltige Nutzung der Biodiversität von Obstarten gemäßigter Klimazonen. *Die Biodiversität von Obstarten gemäßigter Klimazonen hat in den letzten Jahrzehnten in vielen Anbaugebieten der Welt abgenommen. Aber auf der Grundlage des Übereinkommens über die biologische Vielfalt (Convention on Biological Biodiversity; CBD) und des Globalen Aktionsplans (Global Plan of Action; GPA) haben mehrere Länder ihre Bemühungen um die Erhaltung und nachhaltige Nutzung pflanzengenetischer Ressourcen verstärkt. In der Schweiz wurden erhebliche Fortschritte bei der Erkennung, Erhaltung und Charakterisierung von genetischen Ressourcen bei Obstarten erzielt. Richtlinien für die phänotypische Beschreibung dieser genetischen Ressourcen wurden entwickelt und angewendet. Darüber hinaus wurden molekulare Marker eingesetzt, um Duplikate zu erkennen und so Genbank-Sammlungen zu rationalisieren.*
Schlagwörter: Biodiversität, genetische Ressourcen von Obstarten, Feuerbrand, Fruchtqualität

Biodiversity has become an important global issue. The United Nations declared 2010 to be the International Year of Biodiversity. The world was invited to take action to safeguard the variety of life on earth: biodiversity. The Convention on Biological Diversity (CBD) was a key agreement adopted at the United Nations Conference on Environment and Development that was held in Rio de Janeiro in 1992 (www.biodiv.org). The convention has fostered international activities for the conservation and sustainable use of plant genetic resources for food and agriculture. The three main goals: the conservation of biological diversity, its sustainable use, and the sharing of benefits from their use. The conservation of plant genetic resources (PGR) has gained significantly in importance and is now accepted as an essential responsibility of national governments (Engels, 2002). Based on the CBD a Global Plan of Action for the Conservation and Sustainable Use of Plant Genetic Resources for Food and Agriculture was established in Leipzig in 1996 (www.fao.org). The organization 'Bioversity International' (the former IPGRI, International Plant Genetic Resources Institute) with its headquarters in Rome, Italy, is seeking to advance the conservation and use of plant genetic diversity for the well-being of present and future generations worldwide. The European Cooperative Program for Plant Genetic Resources (ECPGR, www.ecpgr.cgiar.org) is a collaborative program including most European countries and is aimed at facilitating the long-term conservation and increased utilization of plant genetic resources in Europe. It was founded in 1980 and is financed by the member countries and coordinated by 'Bioversity International'. The ECPGR Documentation and Information Network has contributed to making information on ex situ conserved germplasm available. As

a result of an EU-funded project and ECPGR support, the EURISCO catalogue (http://eurisco.ecpgr.org/) provides on-line passport information on accessions conserved in European collections. The ECPGR fruit network comprises working groups on *Malus/Pyrus*, *Prunus* and *Vitis*. Special central databases for each species were established (for apples see: http://www.nationalfruitcollection.org.uk/ecpgr.php) and currently a European *Malus/Pyrus* collection is being established. As in many crops, the genetic diversity in fruit species has considerably decreased in recent decades and a few fruit species and cultivars grown worldwide have become predominant. ECPGR decided to establish an efficient and rational European conservation system, with an initial focus on existing ex situ genebank collections in European countries. The goal is to create A European Genebank Integrated System (AEGIS) aimed at conserving the genetically unique accessions for Europe and making them available for breeding and research. Such material will be safely conserved under conditions that ensure genetic integrity and viability in the long term (KELLERHALS, 2010).

The United States Department of Agriculture (USDA) Agricultural Research Service (ARS) maintains an apple germplasm collection in Geneva NY that includes more than 8500 accessions representing at least 50 species. Of these, some 2600 accessions are cultivars, 3100 are seedlings mainly representing species collections, 1600 are in the form of seed, and 1250 are wild by elite hybrids that were generated specifically for genetic studies (SIMON et al., 2008). The purpose of this collection includes the acquisition, maintenance, characterization and distribution of the diversity of *Malus.* The collection was primarily characterized with 154 descriptors including pomological, pathological, anatomical and physiological characteristics. They are recorded in the Germplasm Resources Information Network (GRIN; www.ars-grin.gov). A set of microsatellite markers was included and the data are publically available. Similar collections exist in the US for other temperate fruit species.

Conservation, description and use of fruit genetic resources in Switzerland

In close collaboration with NGOs the Swiss government is supporting and implementing structures for a sustainable conservation and utilization of plant genetic resources. The national campaign plant (NCP) is coordinated by the Swiss Commission for the conservation of plant genetic resources (www.cpc-skek.ch). Financial support for the commission and for specific projects is given on a grant basis by the Federal Office of Agriculture. KELLERHALS and EGGER (2004) reported about the field inventory carried out to detect as many still existing Swiss fruit genetic resources as possible. This inventory allowed subsequently a complete and safe conservation of fruit genetic resources. 2800 accessions were selected for long-term conservation or further evaluation. They were planted, either in Primary Collections for the long-term conservation or into Introductory Collections for further phenotypic and molecular evaluation. The conservation of fruit genetic resources is organized in a decentralized way with collections spread predominantly over the northern part of Switzerland. Every accession is planted in at least two different collections in order to prevent loss of genetic material.

Following the inventory, a project for the characterization of the rich fruit genetic resources was initiated in order to allow for their use in fruit-growing and in breeding. Both the inventory and the characterization projects were running under the responsibility of the NGO Fructus (www.fructus.ch) in collaboration with many other stakeholders. Around 800 accessions were described and scientifically photographed within the characterization project so far. Further evaluation of selected accessions included the production and evaluation of 480 fruit juice samples and 180 fruit liquor samples, the testing of 600 apple accessions for their scab and mildew susceptibility in the field, the testing of 160 apple and pear accessions for their fire blight susceptibility in the quarantine glasshouse and the genetic characterization of 1037 apple and 452 cherry accessions with SSR-markers. Fire blight is one of the most serious diseases and causes great losses in pome fruit cultivation. It is also a threat for genetic resources collections (PEIL et al., 2004).

Among the *Malus* genetic resources a broad range of resistance levels towards fire blight can be found (ALDWINCKLE et al., 1976). Since fire blight first appeared in Switzerland in 1989, it has steadily spread from the original epicentre in the north-eastern cantons to the south-western regions (DUFFY et al., 2005). In 2007, the so far most significant outbreak took place and several of the decentralised genebank collections suffered from losses.

Materials and methods

Description of fruit genetic resources

Guidelines were developed for the coordinated description of the fruit genetic resources in Switzerland (SZALATNAY, 2006). These descriptors are for the most part consistent with the ECPGR descriptors and also considering UPOV descriptors. They include a total of 175 fruit and 62 tree characteristics for apple, pear, sweet cherry and plum. Most of the descriptors are illustrated with color pictures. Every year about 500 accessions were described according to these guidelines and the data are included in the Swiss National Database for Plant Genetic Resources.

Evaluation of fire blight susceptibility

The screening of heritage varieties for relative fire blight tolerance was conducted in the quarantine glasshouse at the Research Station Agroscope Changins-Wädenswil ACW in Wädenswil, where brand-new facilities are available since 2010. Scion material was grafted onto 'M9' rootstocks. In spring trees were planted in plastic deep-pots 60 (Stuewe & Sons, Corvallis, US) with a length of 35.5 cm and a diameter of 7 cm and then grown in the glasshouse for several weeks prior to inoculation. For each variety, 6 to 10 replicate trees were inoculated by puncturing the distal tip of shoots 15 to 30 cm long with a syringe containing an *E. amylovora* solution of 10^9 cfu/ml strain FAW611. Disease progress was evaluated weekly for three weeks by measuring the expansion of the necrotic lesion from the shoot tip in relation to the total shoot length. Artificial fire blight infections were performed in 2007 and 2008 each with 40 and in 2010 with 80 different apple and pear accessions, including heritage varieties still grown for apple juice and cider production, and with commercial standard varieties. Most of these accessions were also analysed for the presence or absence of molecular markers flanking a fire blight resistance QTL which was described by CALENGE et al. (2005) and KHAN et al. (2007). The two SCAR markers AE 10-375 and GE-8019 were used to spot accessions that carry both of these markers and conclusively also the fire blight resistance QTL.

Fruit quality

A broad fruit quality analysis of apple accessions was performed in 2007 at harvest time with the Pimprenelle robotic machine. It measured firmness, sugar and acidity. Further testing included processed products of fruit samples. A total of 480 fruit juice samples (apple and pear) and 180 fruit liquor samples (apple, pear, cherry, plum) were produced and evaluated between 2007 and 2010.

Fruits samples of about 20 kg each from the selected varieties were collected in the respective fruit tree collections or from growers. Fruits were picked by hand to avoid damage before processing. A pack press was used to receive the juice applying a pressure of 280 bar over 5 minutes. The juice was clarified by adding a pectinase Ultrazym 100 (5 mg/l) and gelatine (5 % solution, 1 ml/l). About 12 hours later the juice was filled into 0.5 l-bottles with a crown cap and pasteurized at 78 °C. Analysis of the fruit juices comprised total sugar, titratable acidity and antioxidative potential. Besides total sugar content also different sugars such as glucose, fructose, and saccharose as well as the sugar alcohol sorbite were analysed.

Results

Description

A handbook with guidelines for the coordinated description of fruit and tree characteristics was developed (SZALATNAY, 2006). Many descriptors which are continuously variable are recorded on a 1 to 9 scale.

The description of apple, pear, plum and cherry accessions is under way but not yet completed. Data are continuously integrated in the Swiss National Database (www.bdn.ch).

Fire blight

The results of fire blight testing in the glasshouse revealed a wide range of tolerance and susceptibility among the apple and pear accessions tested. Figure 1 shows results with 30 accessions tested in 2008. The apple variety 'Schneiderapfel' was highly resistant in our trials. But 'Schneiderapfel' is a triploid cultivar and can therefore hardly be used in breeding. Other cultivars such as 'Ohio Reinette' and 'Danziger Kantapfel' were already used in crosses to develop new apple cultivars with high fruit quality and increased fire blight tolerance. Comparison of the fire blight test results with the same accessions performed in 2007 and 2008 revealed a satisfactory reliability of the test, although two different *E. amylovora* strains had been used (Fig. 2). Further tests are needed to confirm these results and they will contribute to find outstanding accessions among

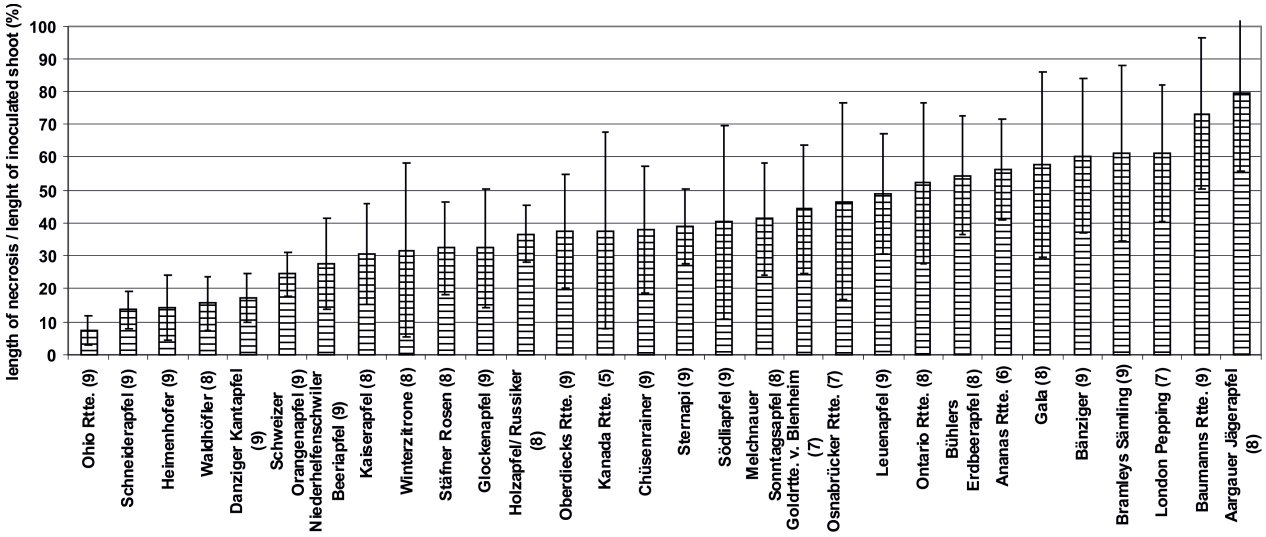

Fig. 1: Fire blight glasshouse screening of 30 traditional apple cultivars with 'Gala' as standard, year 2008 (scoring 3 weeks after inoculation, number of plants in brackets, bars represent standard deviation)

the Swiss fruit genetic resources.

Both SCAR markers AE and GE and therefore the resistance QTL was present in four out of 39 tested accessions, namely 'Dettighofer', 'Bernecker Wildling', 'Schweizer Orangenapfel' and 'Sternapi'. These accessions had an average necrosis length of 39.8 % of the total shoot length compared to 44.5 % for the average of the accessions carrying none ore only one of the flanking SCAR markers.

Fruit quality in fresh and processed accessions

To gain a rough overview on selected internal fruit quality parameters at harvest time, more than 600 apple

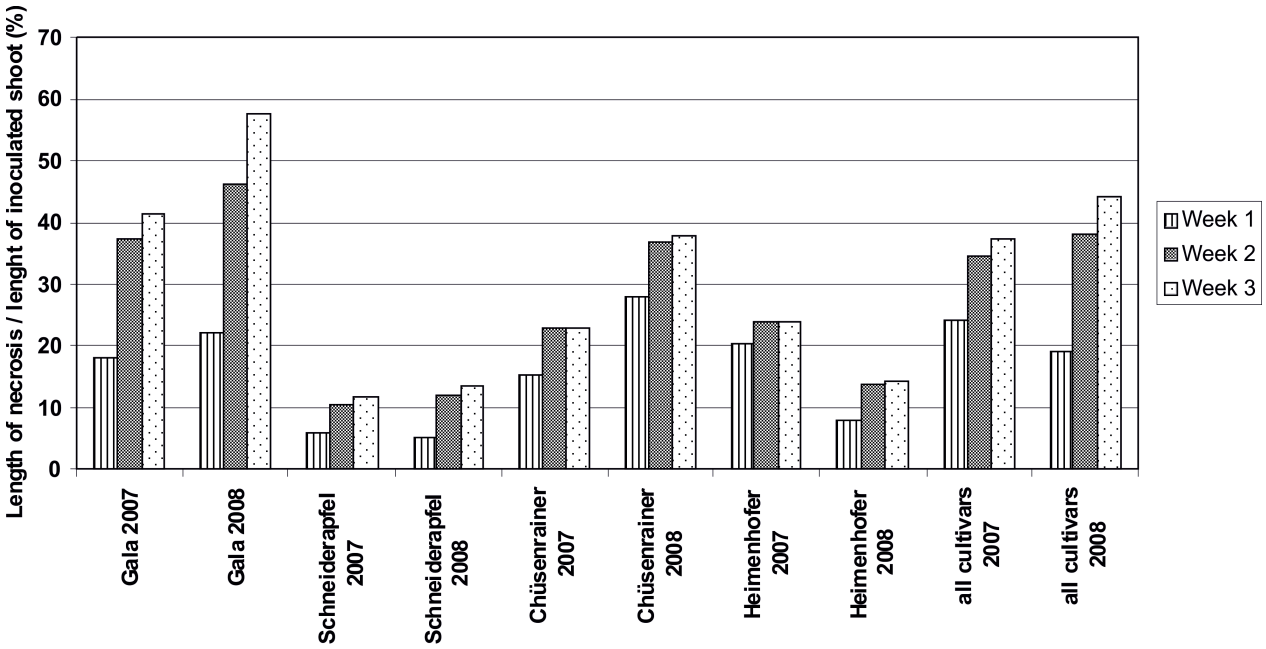

Fig. 2: Comparison of fire blight glasshouse screening with selected cultivars tested in 2007 and 2008 (scoring 1, 2 and 3 weeks after inoculation)

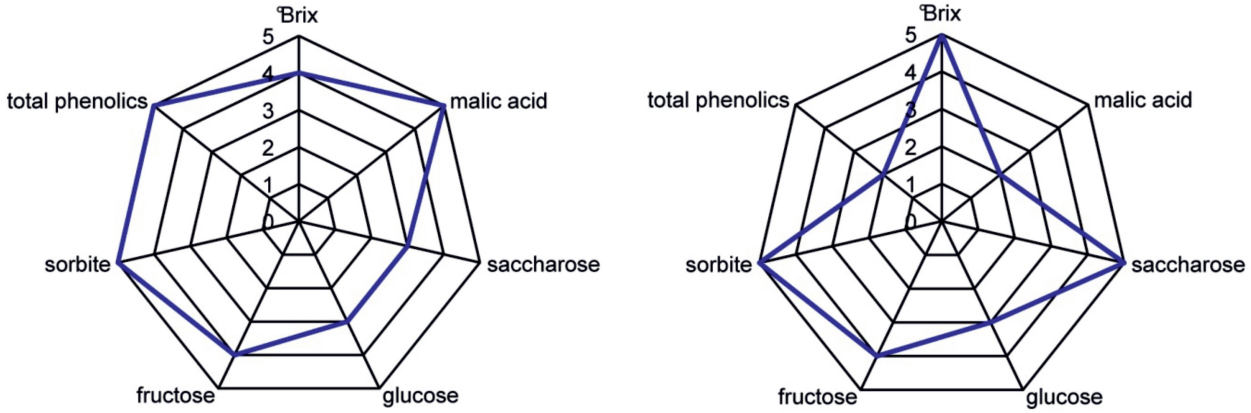

Fig. 3: Comparison of the analytical data from juices of the apple varieties 'Engishofer' (left) and 'Wildmuser' (right)

samples were tested in 2007 with the Pimprenelle robotic machine. The results revealed a wide variety of firmness as well as sugar (°Brix) and malic acid contents (g/l) at picking time. Additionally to the tests with the Pimprenelle robotic machine, fruit juice samples from specific accessions are being produced for instrumental and sensory testing by a panel of experts.

Interpretation of the analytical data originating from true-to-type varietal fruit juices reveals considerable differences between accessions. For example the juice of the apple variety 'Engishofer' displayed a very high content of total phenolics and malic acid whereas the variety 'Wildmuser' was high in sugar but low in acidity and total phenolics (Fig. 3).

For the sensory evaluation a professional scoring system of the Swiss Quality Competition for Apple Juice was used. In 2009 almost a quarter of all juices reached a score of at least 17 points. Besides many unknown local heritage varieties there were also a range of well-known varieties such as the apple variety 'Jonathan'. This variety, non-indigenous to Switzerland, regularly achieves high scores in juice competitions. This confirms the fairly good reproducibility of the sensory scoring. Other highly rated juices originated from the typical old Swiss varieties 'Heimenhofer' and 'Sauergrauech'. Although rated lower, other cultivars with high acidity are potentially interesting as they might be desired to equilibrate juices mainly derived from table fruit with high sugar content and low acidity.

Similarly liquors from 180 different accessions were distilled and evaluated by an expert panel. In 2009 there were 16 cherry and 24 pear liquors distilled. As for the cherries there were on average 7.9 kg of fruit and the recovery was 5.2 %. Among these distillates there were significant differences in quality. The sensory evaluation by the experts highlighted the distillate of the variety 'Inselkirsche' (ID 67776) as especially typical, spicy and intensive and with a score of 18 as the best one. The 'Buschelkirsche' (ID 55140) and the 'Kirschmuskirsche' (ID 61034) were also highly appreciated and reached 17 points. Another 5 accessions reached 16 points.

As for the pears, on average 12.5 kg of fruit were mashed and a recovery of 4.5 % was obtained. With the pears there were two unnamed accessions that reached a very good result with a score of 18 points, another three reached 17 points.

Discussion

The characterization of fruit genetic resources in Switzerland by using well-established guidelines for the description and including tests related to disease resistance and fruit quality shows promise for their exploitation in fruit growing and breeding.

The information on the differential resistance and susceptibility of the Swiss apple and pear genetic resources towards diseases is important for breeding and for replanting traditional varieties. This will enable informed decision making as to which varieties to replant and which to avoid due to fire blight susceptibility. Further tests are needed to confirm these results under natural disease pressure especially related to flower infection with the fire blight bacteria.

The results of the fruit quality testing help to find accessions of specific interest, either for breeding or special processing purposes. A similar approach was followed by Toth et al. (2004) in Hungary. A main focus in

Switzerland will be to identify accessions with both high acid and sugar contents for the fruit juice industry. These projects deliver a deep insight into the variation and abundance of a wide range of tree and fruit characters in apple, pear, cherry and plum accessions kept in Swiss genebank repositories. The results can be exploited in breeding programmes, for practical fruit growing and for the production of high quality juice and liquor products.

It is difficult to gain a thorough view on the situation of temperate fruit genetic resources conservation, characterization and use worldwide. In Europe there is, thanks to the ECPGR programme, a collaboration among most countries aimed at facilitating the long-term conservation and the increased utilization of plant genetic resources. In Russia, Pavlovsk Experiment Station near St. Petersburg is one of the oldest centers of horticulture. The buildup of the large fruit collection was started under the Russian botanist Nikolai Vavilov in 1926. Actually, the collection maintained consists of over 5700 accessions of various fruit, berry and ornamental plants, including 634 accessions of apple, 75 of pear and 320 of plum, cherry and other *Prunus* species. But due to financial constraints this collection is endangered to be removed and there is considerable concern about its future. In several eastern European countries there are difficulties to maintain their fruit germplasm collections as well. Even in Western Europe due to restructuration of institutes and financial constraints the safe long-term conservation of fruit genetic resources is not always fully assured.

In the USA, the National Germplasm Repository for Apple in Geneva, USA is part of the National Plant Germplasm System and maintains presumably the largest collection of apple germplasm worldwide, nearly 4000 accessions. Many of these genotypes were collected from the apple centre of diversity in Central Asia (Forsline et al. 2003). As a preventive measure against field disasters, large portions of the national Canadian and USDA *Malus* collections are also backed-up in liquid nitrogen vapour at the USDA-ARS National Center for Genetic Resources Preservation (NCGRP) in Fort Collins, Colorado (Volk et al. 2008).

There is still little molecular knowledge available on the genetic diversity of fruit genetic resources. The richness of this diversity in terms of useful genes is invaluable and yet to be understood and explored. Increasingly molecular markers such as microsatellites (SSR markers) are used to fingerprint fruit germplasm. Evans et al. (2009) established within ECPGR a standard set of robust microsatellites to allow comparisons between *Pyrus* germplasm collections. Similar sets were defined by Clarke (2009) for sweet cherry and will be established for further species. On a collaborative workshop the ECPGR *Malus*/*Pyrus* Working Group agreed upon a standard set of eight reference accessions and 12 SSR markers for molecular fingerprinting of apple (Van Teuren et al. 2010). Using the standard sets for fingerprinting reliable comparisons can be made between laboratories and datasets, and collections can be screened cost-effectively.

Frei et al. (2010) described the molecular characterisation of the national collection of Swiss sweet cherry cultivars. 567 sweet cherry accessions were analysed. Of the 20 SSRs tested, 14 resulted in polymorphic and useful multiplex mixes covering the entire cherry genome. Several groups of putative synonyms, with up to 16 accessions, were found, reducing the number of individual genetic profiles to 278. This represents a 49 % reduction of the total number of genetically different accessions. The fact that almost 50 % of all Swiss sweet cherry accessions were synonyms significantly reduces the total number of unique accessions in the germplasm collections, resulting in a reduced planting area, enhanced maintenance efficiency and thus reduced overall cost for maintaining the collections.

The long-term storage of germplasm under cryogenic conditions is an efficient approach (Towill et al., 2004). The USDA-ARS National Center for Genetic Resources Preservation (NCGRP) routinely cryopreserves *Malus* dormant buds by first desiccating single bud sections to 25 to 30 % moisture content, slow-cooling tubes of buds sections at 1 °C to -30 °C, holding the samples at -30 °C for 24 h, and then quickly placing tubes into liquid nitrogen vapour for long-term storage (Forsline et al., 1998). While the active collection focuses on germplasm maintenance, characterization, documentation and distribution to the user community, the base collection provides long-term preservation and is distributed only when needed for reproduction at the active site. The USDA-ARS, Plant Genetic Resources Unit at Geneva, NY (USA) keeps 1915 accessions of *Malus* under cryopreservation. The method was successful with 91 % of the lines tested having viability after cryo-exposure of at least 40 %. Genotypic differences in survival were observed and the success varied from year to year. While genomics and bioinformatics are essential tools to understand and exploit variation of genes, genomes and genepools, cryopreservation with its minimal requirements of space and mainten-

ance continues to gain importance as a means of long-term physical storage of genetic materials (FORSLINE et al., 2003). In Germany, for building a backup cryopreservation, the method using winter vegetative buds was successfully applied to the first *Malus* accessions maintained at the Julius Kühn Institute JKI genebank (HÖFER, 2007).

Genetic diversity provides the raw material for breeding and plant improvement. It allows to react to new requirements of the consumers and markets as well as to climate change.

Acknowledgements

We acknowledge financial support for the relevant projects contributing to this paper by the National Campaign Plants (NCP), a Swiss Federal Office for Agriculture's program and for the collaboration with the NGO Fructus (www.fructus.ch) and all other project partners and collaborators.

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