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THE CORE COLLECTION OF THE NORTHERN EUROPEAN GENE POOL OF *RIBES* CREATED BY RIBESCO PROJECT

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The RIBESCO project, partly funded by the European Community, has been designed to improve the level of characterisation and conservation of the Northern European *Ribes* germplasm, currants and gooseberry, utilising coordinated, international achievements. A core collection of the Northern European *Ribes* germplasm will be established and preserved with special intensity. In the first phase, the *Ribes* germplasm conserved in the national *ex situ* collections is subjected to phenotypic characterisation using internationally defined descriptors. Information obtained will be saved in a database and it will be used to select material to the *Ribes* core collection and to define the part of germplasm that will be identified using molecular markers. The data of molecular identification provide a suggestion for an optimal core collection, representing a maximum amount of genetic variation, with true-to-type varieties selected and duplicated accessions uncovered. The selection of the most valuable genotypes is also based on agronomic, historical or other important cultural values. The decentralised *Ribes* core collection is established to assure the preservation of selected germplasm, each plant accession to be conserved as safety duplicates. For conservation, both *ex situ* field collections, *in vitro* slow-growth collections and cryopreservation long-term collections are established. Easy-access documentation will be a part of the activity. The project promotes *Ribes* genetic material transfer and use on a whole European scale.

Key words: cryopreservation, currants, database, gene bank, germplasm, gooseberry, markers.

Introduction. Northern Europe is the leading production area of *Ribes* fruits (Lanham et al., 1995). *Ribes* species are also known by their high level of active ingredients available for functional and healthy-food products (Tahvonen et al., 2005; Johnston et al., 2007; Pantelidis et al., 2007; Wu et al., 2007). The genus *Ribes* represents an important part of small fruit germplasm in the Northern European gene bank collections, but at present there is no trans-national coordination of these collections.

Due to the long history of domestication and cultivation of *Ribes* species (Hjalmarsson and Wallace, 2007), the national gene bank collections in the northern European region consist mostly of old and found varieties, with possibly several synonyms and even wrongly-named accessions. In addition, varieties have been renamed, possibly several times, and on the other hand varieties mutated but still named similarly may be included in the collections. The collections also contain several unnamed accessions.

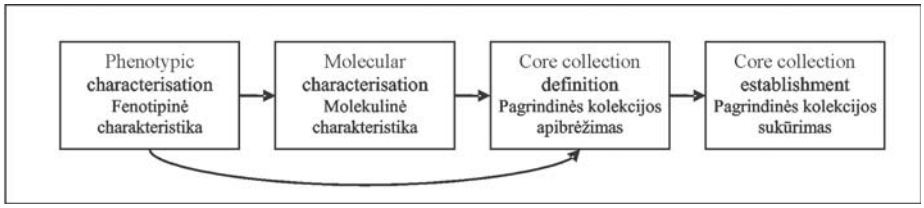
During the last few years, dynamic developmental processes in the European agricultural research field have been experienced, including several institutional changes. These changes have also affected national plant archives, gene banks, adding pressure to limit and rationalise the germplasm preservation activities. This has especially concerned the collections of those horticultural plants that cannot be preserved as seeds but must be maintained in *ex situ* collections as plants. In addition, the on-farm conservation of clonally propagated material is risky, because the plants can be replaced by other, newer varieties.

In Northern Europe, both black and red currant species are grown wild, covering a valuable gene pool with high winter and spring frost tolerance, late blooming and a short fruit development phase (Lanham et al., 1995; Brennan et al., 2007). Collections of these wild or early-domesticated genotypes have been achieved in the past, but comprehensive documentation and conservation of the plant clones has not been organised with safety back-up collections. So far, there is no common coordination system of the *Ribes* germplasm collections. Under the European Cooperative Programme for Plant Genetic Resources (ECPGR), the European Central Ribes/ Rubus Database has been created, but its development has been discontinued.

A four-year project, *RIBESCO – Core collection of Northern European gene pool of Ribes,* was launched in April 2007 to improve the level of characterisation and conservation of Northern European *Ribes* germplasm utilising coordinated, trans-national achievements. The project is funded half-and-half by the participating institutes using their national financing and by 'the Community programme on the conservation, characterisation, collection and utilisation of genetic resources in agriculture' established by the European Commission (EC).

The core collection does not exclude the possibility to maintain also larger, e.g. field collections, but plant breeders seldom access a diffuse germplasm collection. For breeding work a well-documented core collection offers a more efficient and targeted gene pool than diffuse large germplasm collections. The *Ribes* core collection thus promotes the utilisation of this germplasm.

Approach and structure of the project. The RIBESCO project will improve the level of characterisation and conservation of the Northern European *Ribes* germplasm (currants and gooseberry) by coordinated implementation. The information and safety level of the collections of *Ribes* genotypes is raised, and the high quality of the collection is assured.



F i g. The structure of the project
P a v. Projekto struktūra

The structure of the project is progressive, one stage leading to another, with some parallel actions (Fig. 1). A trans-national network is created, combining both scientists and breeders working on northern *Ribes* germplasm. Both phenotypic characterisation and national knowledge as well as molecular identification are utilised to define the core collection. The project leads to the increased conservation security and documentation of the most valuable part of *Ribes* germplasm in the northern area of the EC. The participating institutes are MTT Agrifood Research Finland (as coordinating partner), Estonian University of Life Sciences, The Research Institute of Pomology and Floriculture (Poland), Swedish University of Agricultural Sciences, Vilnius University (Lithuania), Lithuanian Institute of Horticulture, Bundessortenamt (Germany), Latvian State Institute of Fruit Growing, and The University of Copenhagen (Denmark).

Phenotypic characterisation. The first phase of the project is focused on the phenotypic characterisation of *Ribes* gene bank material conserved in *ex situ* collections. This characterisation is done by employing traditional practices, observation, measurement and documentation of plant traits. The primary characterisation serves also as a screening for microsatellite analyses, to keep the expenses of molecular studies at a moderate level.

Germplasm is evaluated for a number of characteristics. These characteristics include both agronomic traits – such as crop earliness and winterhardiness – but also more neutral, morphological characters. The characteristics are of heritable quality and only marginally affected by the environment. Because the present and most possible future use of *Ribes* species is based on fruit production, also several fruit quality factors, such as colour and even taste, contributes are evaluated. Keeping in mind the special needs of sustainable production methods, pest and disease resistance, for instance to mildew (*Shaerotheca mors-uvae*), is also evaluated.

Characterisation is designed to suit in the best possible way to the documentation of the genetic traits of the plant material in the gene bank collections. It is based on 'the Guidelines for the Conduct of Tests for Distinctness, Homogeneity and Stability' by the International Union for the Protection of New Varieties of Plants (UPOV),

offered separately for black and red currants and gooseberry. The additional evaluation criteria created by the Nordic Gene Bank and accepted by the Nordic and Baltic countries are also applied and modified when necessary.

Data collection and recording is harmonised in a way that it can be saved in a consistent database and then be transferred to the European Central *Ribes* Database of ECPGR. The phenotypic characterisation will improve the documentation level of collections and, as a consequence, the accessions will have a greater value for instance in breeding work.

Molecular characterisation. Molecular analyses are designed to give a reliable estimate of the genetic relationships among the candidate varieties for the core collection. The molecular data provide a suggestion for an optimal core collection, which would represent a maximal amount of genetic variation. It also assures the trueness-to-type of varieties to be selected to core collections and uncovers duplicated accessions.

In recent years a substantial amount of molecular markers have been developed and tested to accurately reveal polymorphism in *Ribes* (Lanham and Brennan, 1998; Lanham and Brennan, 1999; Lanham et al., 2000; Brennan et al., 2002; Brennan et al., 2007). The first objective is to standardise and calibrate microsatellite marker analysis methods (SSR, Simple Sequence Repeats and ISSR, Inter-Simple Sequence Repeats) for the cultivated *Ribes* species in the participating laboratories. The calibration of the methods is a prerequisite for the undependable comparison of the results from each partner's analysis of their own candidate genotypes. The partners will first employ the previously selected primers in the analysis of the standard set of genotypes. The results will show what kind of adjusting is needed to make the results generally comparable. After the calibration phase, all the partners analyse their own group of candidate genotypes. The aim is to provide molecular marker data of all candidates of the core collection. Thereafter, estimates of genetic relationships among the candidate genotypes are provided, and an optimal group containing a maximum amount of genetic variation is defined.

Core collection definition. The most valuable *Ribes* germplasm is selected to a core collection that will include maximal genetic variation with a minimum of repetitiveness. The core collection candidates are defined partly on the basis of the data of phenotypic characterisation. In addition, the selection is partly based on agronomic, historical or other important cultural values. This evaluation of candidate genotypes is done both at national and international level. Each nation defines which part of the collection is important from the national point of view. In addition, the collected data are considered as a whole, and the selection of candidates is done at the level of all genetic material. This pre-core collection is estimated to contain 25% of the material of collections, varying according to the size of collections and the type of plant material (known varieties, land races or unidentified material).

The final definition of the core collection is made by combining the results of phenotypic characterisation and agronomic, historical or other important cultural values with the microsatellite analysis data. A part of the germplasm can be categorised to belong to the core collection on the basis of passport data only, if, for instance, the cultural value of an old and verified variety is unarguable. In the same way as

selecting the core collection candidates, the final selection is done both at national and international level; the genetic material is considered as a whole, but the national standpoints are taken into account. It is estimated that approximately 10% of all analysed genotypes will be included in the core collection.

Core collection establishment. Collection standards. Standards for the quality of *Ribes* core collection are established in accordance with the existing national guidelines and the international guidelines of the International Plant Genetic Resources Institute (IPGRI) (Reed et al., 2004). This includes specifying among other things the standards for location, planting conditions, minimum level of care, number of plants per accession, documentation follow-up, utilisation and renewing of the collections.

The safety of germplasm collections requires duplication at another site. Different storage methods will be used. The main conservation method for *Ribes* germplasm is maintaining plants in *ex situ* field collections. *In vitro* collections are needed both as duplicate storages and as temporary collections for the accessions to be cryopreserved. In long run, cryopreservation will be the objective in the long-term duplicate preservation of *Ribes* genetic resources. Duplication can be a combination of field plantings, *in vitro* culture and cryopreservation. The participating institutes will agree on standard procedures for handling the plant material.

Establishment of field collections. Either new plantings or collections already established can serve as core collection, if they fulfil the standards. Proper site is an important factor in maintaining the health of the collection and in minimising the potential environmental hazards. The plants of the core collection can be grown in greenhouses, screen houses and in the field. The plants are propagated vegetatively by cuttings. This method can most likely be applied to all *Ribes* genotypes. Also micropropagated material can be used. Plant health must be considered at establishing the collection. Propagules shall be taken only from a healthy looking stock and if virus free material is available, it should be used.

Establishment of *in vitro* collections. Also *in vitro* collections can provide the long-term conservation method of core collection genotypes. The advantage of establishing *in vitro* cultures through meristem culture is virus elimination at least in some degree. *In vitro* maintenance can be carried out using methodology established to *Ribes* species (Welander, 1985; Orlikowska et al., 1991). *In vitro* storage may be in warm or cool conditions. The selection of the method will depend on the genotype and the available techniques. Slow-grow storage in cool conditions decreases labour requirements, costs and chance for contamination of cultures and the risk of loosing germplasm through different handling errors. It also decreases the risk of genetic instability.

Establishment of cryopreservation collections. Also cryopreservation can be applied in some laboratories for long-term preservation of the selected germplasm (Wang et al., 2005; Johnston et al., 2007). This material shall first be established *in vitro*. One major advantage of cryopreservation is that it can also be used for virus eradication. Cryopreserved meristems are stored in liquid nitrogen at –196°C or in the gas phase of it below –150°C.

Benefits and future prospects. The main target of the RIBESCO project is to assure the high-level conservation of genetic variability of *Ribes* species. This leads to several benefits. Both the starting point and the one major objective of the RIBESCO project are to establish the collaboration of the national agents responsible for the conservation of *Ribes* germplasm. Creating the core collection and improving the data availability can more effectively direct national resources to conservation, when overlapping resource allocation is removed.

Improved data availability also ensures easier access and utilisation of the collections. Breeding work and product development, for instance, will benefit from that (Šiksnianas et al., 2006). The achievements of the project will also improve the quality of collections. The level of characterisation is increased, and documentation will include the data of molecular identification. The project thus also increases the level of scientific information on the collections. This will add the value of the collections, and increase the exchange and utilisation of this genetic resource.

Although the RIBESCO project includes the Northern pool of European *Ribes* germplasm only, this genetic resource is also valuable to more southern European regions. The improved availability of data will also benefit these areas. The conservation of more southern genetic resources of *Ribes* can be developed accordingly, by adapting the operational mode applied in RIBESCO. The structure created in this project is also open to be expanded, finally to cover the whole European area.

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PROJEKTO "RIBESCO" SUKURTA BENDRA ŠIAURĖS EUROPOS *RIBES GENTIES* GENOFONDO KOLEKCIJA

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Santrauka

Projektas "RIBESCO", iš dalies finansuojamas Europos Bendrijos, yra skirtas pagerinti ir išsaugoti Šiaurės Europos *Ribes* genties, serbentų ir agrastų, genofondo charakteristikas, panaudojant suderintus tarptautinius pasiekimus tarp mokslo institucijų. Bus sukurta ir saugoma bendra Šiaurės Europos *Ribes* genties genofondo kolekcija. Pirmame etape bus atliekamas fenotipinis *Ribes* genties genofondo, esančio nacionalinėse *ex situ* kolekcijose, charakterizavimas, panaudojant tarptautinius deskriptorius. Gauta informacija bus saugoma duomenų bazėje ir panaudojama atrenkant medžiagą bendrai *Ribes* genties kolekcijai bei apibūdinant tą genofondo dalį, kuri bus identifikuota pritaikant molekulinius žymenis. Molekulinės identifikacijos duomenys sudarys galimybes sukurti optimalią bendrą kolekciją, reprezentuojančią maksimalų genetinių variacijų skaičių, su atrinktomis tipiškomis veislėmis ir išaiškintais dublikatais. Vertingiausių genotipų atranka bus pagrįsta taip pat agronominėmis, istorinėmis bei kitomis svarbiomis savybėmis. Bus sukurta bendra *Ribes* genties kolekcija, siekiant išsaugoti atrinktą genofondą. Bus sukurtos *ex situ* lauko kolekcijos, *in vitro* kolekcijos ir žemų temperatūrų metodu išsaugomos ilgalaikės kolekcijos. Vienas iš projekto tikslų – sukurti lengvai prieinamą dokumentaciją. Šis projektas skatina *Ribes* genties genetinės medžiagos perkėlimą ir panaudojimą Europiniu mastu tarp mokslo institucijų.

Reikšminiai žodžiai: agrastai, duomenų bazė, genų bankas, genofondas, serbentai, žymenys.