



Novel characterization of crop wild relative and landrace resources as a basis for improved crop breeding



ISSN 1742-3694 (Online)

www.pgrsecure.org

Crop wild relative

Issue 10 February 2015

PGR Secure – EU Seventh Framework Programme, THEME KBBE.2010.1.1-03, GA 266394



Conserving plant genetic resources

for use now and in the future



Editors:
Shelagh Kell
Nigel Maxted

Design and layout:
Shelagh Kell
Hannah Fielder

Front cover:
Honey bee on *Brassica*
flower

Photo:
Lorenzo Raggi



Crop wild relative

Issue 10 February 2015

Contents

Editorial	3
ENHANCED GENEPOOL UTILIZATION – Capturing wild relative and landrace diversity for crop improvement	
<i>S. Dias, S. Kell, M.E. Dulloo, J. Preston, L. Smith, E. Thörn and N. Maxted</i>	5
PGR Secure exhibits crop wild relatives and landraces at NIAB Innovation Farm	
<i>S. Kell, L. Frese, M. Heinonen, N. Maxted, V. Negri, A. Palmé, L. Smith, S.Ø. Solberg and B. Vosman</i>	9
Phenomics and genomics tools for facilitating brassica crop improvement	
<i>B. Vosman, K. Pelgrom, G. Sharma, R. Voorrips, C. Broekgaarden, J. Pritchard, S. May, S. Adobor, M. Castellanos-Urbe, M. van Kaauwen, B. Janssen, W. van Workum and B. Ford-Lloyd</i>	12
Successful use of crop wild relatives in breeding: easier said than done	
<i>K. Pelgrom, C. Broekgaarden, R. Voorrips and B. Vosman</i>	15
Two predictive characterization approaches to search for target traits in crop wild relatives and landraces	
<i>I. Thormann, M. Parra Quijano, J.M. Iriondo, M.L. Rubio Teso, D.T. Endresen, N. Maxted, S. Dias and M.E. Dulloo</i>	16
Europe's crop wild relative diversity: from conservation planning to conservation action	
<i>S. Kell, B. Ford-Lloyd and N. Maxted</i>	19
Discovering Finnish crop wild relative diversity and gaps in their conservation	
<i>H. Fitzgerald and H. Korpelainen</i>	21
<i>In situ</i> conservation of CWR in Spain: present and future	
<i>M.L. Rubio Teso, C. Ronquillo Ferrero, A. Nebreda Trejo, M. Parra Quijano, E. Torres Lamas and J.M. Iriondo Alegria</i>	24
Italian crop wild relatives and wild harvested plants conservation strategy	
<i>L. Panella, F. Landucci, D. Donnini, D. Gigante, R. Venanzoni, L. Raggi, R. Torricelli and V. Negri</i>	27
Plant Genetic Resources Diversity Gateway for the conservation and use of crop wild relative and landrace traits	
<i>S. Dias</i>	29
<i>Hordeum vulgare</i> subsp. <i>spontaneum</i> in Turkey: characterization in a laboratory environment	
<i>F. Gürel, C. Uçarlı, E. Yörük and A. Karagöz</i>	31
Enhancing the conservation and use of <i>Medicago</i> genetic resources by means of targeted collection using next-generation sequencing	
<i>H. Fielder, B. Ford-Lloyd and N. Maxted</i>	33
On the sustainable use and conservation of plant genetic resources in Europe	
<i>L. Frese, A. Palmé, L. Bülow, G. Neuhaus and C. Kik</i>	34

Correspondence address:

Shelagh Kell, School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK
Email: s.kell@bham.ac.uk

Copyright © University of Birmingham 2015. All rights reserved. The University of Birmingham edits and publishes *Crop wild relative* on behalf of PGR Secure, a collaborative project funded under the EU Seventh Framework Programme, THEME KBBE.2010.1.1-03, Grant Agreement no. 266394

Welcome to Issue 10 of *Crop wild relative*, the final issue to be published under the umbrella of the recently concluded PGR Secure project. In this issue we deliver some of the key highlights and results of the project, starting with an overview of the highly successful joint PGR Secure/EUCARPIA conference, 'ENHANCED GENEPOOL UTILIZATION – capturing wild relative and landrace diversity for crop improvement', which brought together 140 delegates from 42 European and non-European countries to debate current and future conservation and utilization of CWR and landrace diversity (page 5). In addition to disseminating the results of the PGR Secure project and sharing global knowledge on CWR and landrace conservation and use, the conference provided a unique opportunity to showcase CWR and landrace populations in the demonstration plots of the host institute, NIAB Innovation Farm in Cambridge, UK (page 9).

The development of novel characterization techniques for CWR and landraces was one of the focal areas of the PGR Secure project and in this issue we present overviews of the research undertaken and the key results. On page 12, Ben Vosman and colleagues from DLO, The Netherlands report on the development of phenomics and genomics tools to facilitate brassica crop improvement, while on page 15, Koen Pelgrom and colleagues (DLO) summarize the challenges of using CWR in plant breeding programmes but end on a note of optimism that novel techniques will increasingly overcome the problems and lead to an increase in the use of CWR for crop improvement. In combination with population occurrence data, comprehensive environmental datasets which are widely and freely available can be used to characterize populations based on the environmental profile of the sites in which they occur and thus narrow down target populations for characterization using conventional (or novel) phenotypic or DNA-based techniques. Imke Thormann (Bioversity International) and colleagues (page 16) report on predictive characterization approaches tested in the PGR Secure project to search for target traits in CWR, as well as in landraces.

CWR conservation strategy planning was another key objective of the PGR Secure project. On page 19, Shelagh Kell and colleagues (University of Birmingham) highlight the value of Europe's CWR diversity for food and economic security in the region and stress the need for a coherent, regionally coordinated policy and the appropriate resources to fund their conservation, characterization and evaluation. As highlighted in *Crop wild relative* Issue 9 (editorial), national CWR conservation strategies are essential for the effective implementation of an integrated CWR conservation strategy in Europe. Many

countries in Europe have made good progress in developing national CWR conservation strategies (as reported in *Crop wild relative* Issue 9), particularly since the provision of training and support by the PGR Secure project.

However, selected from the north and south of Europe and with known variation in CWR richness and expected differences in the identification of priority CWR, three case studies served to test and illustrate the basic national CWR conservation strategy planning process. On page 21, Heli Fitzgerald (Finnish Museum of Natural History) and Helena Korpelainen (University of Helsinki) summarize the process of preparing the Finland CWR conservation strategy and describe five key CWR sites identified through the application of diversity and gap analysis techniques. Similarly, on page 24 Maria Luisa Rubio Teso (Universidad Rey Juan Carlos, Spain) and colleagues describe how they set about developing a sound evidence-based national CWR conservation strategy for Spain which has one of the largest CWR floras in Europe and contains species of potential major importance for food and economic security in the region. In this article they present the concept of the 'CWR species-ecogeographic unit combination' which combines the application of ecogeographic diversity and complementarity analysis techniques to identify target populations containing potential genetic diversity of adaptive value. On page 27, Lorenzo Panella (University of Perugia) and colleagues describe the production of the CWR inventory of Italy and

present initial results of gap analysis of priority wild relatives in the genus *Brassica*. Importantly, fieldwork undertaken by the authors served to highlight the problems and limitations of basing desk-based conservation planning only on existing occurrence data sourced from herbaria and gene banks, since they discovered that a number of recorded populations could not be found.

On page 29, Sónia Dias (Bioversity International) presents a major product of the PGR Secure project—the PGR Diversity Gateway—which aims to promote and facilitate the use of CWR and landraces in breeding and crop improvement by providing trait and conservation data of potential value to breeders and other users of germplasm, as well as to conservation scientists, policy-makers and other PGRFA stakeholders.

In this issue we also present two guest articles. Filiz Gürel (Istanbul University, Turkey) and colleagues describe the characterization of *Hordeum vulgare* subsp. *spontaneum* (wild barley) accessions for salinity and water stress traits (page 31). The authors stress the importance of identifying and introducing these traits into cultivated barley to mitigate the nega-

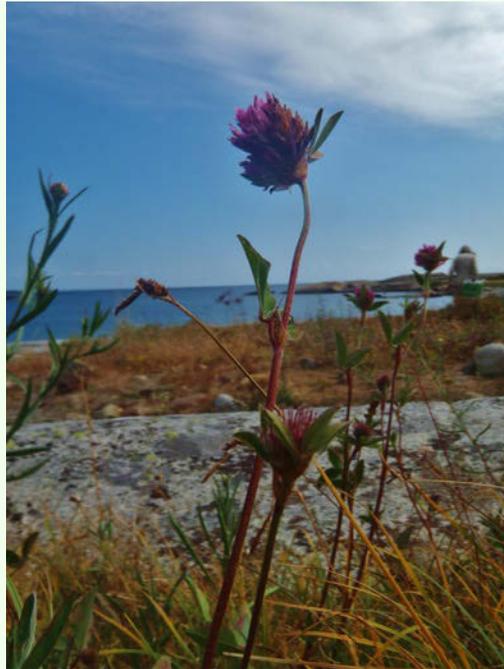


Figure 1 *Trifolium pratense* growing in Færder National park in Vestfold county, Norway (Photo: Jade Phillips)



Figure 2 Sea radish (*Raphanus raphanistrum* subsp. *maritimus*) growing on the Lizard Peninsula in Cornwall, UK (Photo: Hannah Fielder)

tive effects of climatic changes on yield, as well as emphasizing the need for improved efforts to conserve wild barley in Turkey, both *in situ* and *ex situ*. On page 33, Hannah Fielder and colleagues (University of Birmingham, UK) summarize the potential for applying next-generation sequencing to identify gaps in *ex situ* germplasm collections and to aid further collection by targeting genetically diverse samples of CWR harbouring complementary diversity.

One of the key objectives of the PGR Secure project was to address the 'conservation and use divide' by investigating the problems limiting the full and effective conservation and sustainable use of PGRFA collections. On page 34, Lothar Frese (Julius Kühn Institute, Federal Research Centre for Cultivated Plants, Germany) and collaborators present an overview of their extensive work involving stakeholder consultation and SWOT analysis, the results of which are presented in the final report, 'On the sustainable use and conservation of plant genetic resources in Europe' and associated policy paper available at: www.nordgen.org/index.php/en/Plants/Innehaall/Workshops-Conferences/Plant-Genetic-Resource-Workshop-2013/Final-report-and-policy-paper. The authors conclude with 12 actions needed to improve the conservation and sustainable use of plant genetic resources in Europe. In particular, they recommend the establishment of an integrated European Plant Germplasm System which would be composed of sovereign, national plant germplasm system units, and could be coordinated by the European Cooperative for Plant Genetic Resources (ECPGR) with funding from the EU. A bold recommendation to end the PGR Secure project with but one which if left unattended is likely to result in a diminishing and inadequate pool of plant genetic resources for crop improvement, with potentially devastating impacts on European agriculture and on food and economic security in the region.

Another significant success stimulated by PGR Secure has been the beginnings of a putative network of CWR genetic reserves across Europe. Historically, sites that conserve CWR *in situ* have been recognized, but in many cases they are not yet managed in the most appropriate manner to conserve the genetic diversity of the CWR populations included. These sites would not meet the set of quality standards for CWR genetic reserves proposed by Iriondo *et al.* (2012) and their designa-

tion has been *ad hoc* and opportunistic rather than as a result of deliberate scientific selection. Further, they are independent of each other and therefore do not together constitute the desired European network of CWR genetic reserves. However, intensive work has recently been focused on three new sites that do adhere to the Iriondo *et al.* minimum quality standards for CWR genetic reserves:

- Portugal: Ilhéu do Desembarcadouro, a small island in the east of Madeira, and Ilhéu Chão, one of the Desertas Islands located 11 miles southeast of Madeira both contain small populations of the beet wild relative *Beta patula* Aiton. Pinheiro de Carvalho *et al.* (2012) proposed that these sites should be recognized as protected areas with the Natural Park of Madeira specifically to conserve *B. patula*.
- Norway: The Færder National park in southeast Norway will be the site of the first proposed *in situ* CWR genetic reserve in Norway (Fig. 1). The area covered is 340km² of which 15km² is land and home to at least 45 CWR taxa.
- England: The Lizard Peninsula is the most southerly point of the British mainland and home to close to 100 CWR taxa. It is hoped that this CWR rich site will become the first *in situ* CWR genetic reserve in the UK (Fig. 2).

Each of these sites has yet to be formally recognized by the appropriate national authority but it seems likely that each will be formally designated in the near future. Further, the experience of establishing these putative genetic reserves has shown it is possible to bridge the divide between the biodiversity and agrobiodiversity conservation communities, getting the two communities to work together to conserve the genetic diversity of CWR *in situ*.

Finally, although the PGR Secure project has come to a successful conclusion, the consortium of partners involved are actively searching for further funding to continue the development and implementation of conservation and use strategies for CWR diversity inside and outside of Europe. In the mean time we hope you will continue to use the resources available via the PGR Secure website—in particular, the publications page (www.pgrsecure.org/publications), PGR-COMNET (www.pgrsecure.org/PGR-comnet) and the CWR helpdesk (www.pgrsecure.org/helpdesk_cwr)—and of course we are still available to help wherever we can, so please, if you need advice and you think we might be able to help, please contact us.

References

- Iriondo, J.M., Maxted, N., Kell, S.P., Ford-Lloyd, B.V., Lara-Romero, C., Labokas, J. and Magos Brehm, J. (2012) Quality standards for genetic reserve conservation of crop wild relatives. In: Maxted, N., Dulloo, M.E., Ford-Lloyd, B.V., Frese, L., Iriondo, J.M. and Pinheiro de Carvalho, M.A.A. (eds.) *Agrobiodiversity Conservation: Securing the Diversity of Crop Wild Relatives and Landraces*. CAB International, Wallingford. Pp. 72–77.
- Pinheiro de Carvalho, M.Á.A., Nóbrega, H., Freitas, G., Fontinha, S. and Frese, L. (2012) Towards the establishment of a genetic reserve for *Beta patula* Aiton. In: Maxted, N., Dulloo, M.E., Ford-Lloyd, B.V., Frese, L., Iriondo, J.M. and Pinheiro de Carvalho, M.A.A. (eds.) *Agrobiodiversity Conservation: Securing the Diversity of Crop Wild Relatives and Landraces*. CAB International, Wallingford. Pp. 36–44.

ENHANCED GENEPOOL UTILIZATION – Capturing wild relative and landrace diversity for crop improvement

Cambridge, United Kingdom, 16–20 June 2014

S. Dias¹, S. Kell², M.E. Dooloo¹, J. Preston³, L. Smith³, E. Thörn⁴ and N. Maxted²

¹ Bioversity International, Via dei Tre Denari 472/a, Rome, Italy. Email: s.dias@cgiar.org

² School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK. Email: s.kell@bham.ac.uk

³ NIAB Innovation Farm, NIAB, Huntingdon Road, Cambridge, CB3 0LE, UK. Email: lydia.smith@niab.com

⁴ Swedish University of Agricultural Sciences, Genetic Resources and Intellectual Property Rights (GRIP), Division of Legal Affairs, Finances and Human Resources, Box 57, 230 53 Alnarp, Sweden. Email: eva.thorn@slu.se

The international conference, 'ENHANCED GENEPOOL UTILIZATION – capturing wild relative and landrace diversity for crop improvement' (www.pgrsecure.org/conference) was jointly organized by Bioversity International, the University of Birmingham, NIAB Innovation Farm and the European Association for Research on Plant Breeding (EUCARPIA), through its section on Genetic Resources. It was hosted by NIAB Innovation Farm in Cambridge, UK.

The conference was staged to showcase novel characterization techniques and conservation strategies to identify and safeguard CWR and landrace (LR) genetic diversity to increase options for crop improvement as a means of underpinning food security in the face of climate change. It also aimed to disseminate products from the PGR Secure project (www.pgrsecure.org) to the European and global plant genetic resource (PGR) community, and discuss their wider application in the future. The conference brought together a wide range of biodiversity expertise from the international community to debate current and future enhanced conservation and utilization of CWR and LR diversity for improving agricultural production, increasing food security, and sustaining the environment for better livelihoods. The conference represents a landmark in the

science arena of plant genetic resources, highlighting exotic plant germplasm as a critical but neglected resource for crop improvement.

Participation

The conference was attended by 140 delegates from 42 countries, of which half were from outside Europe, confirming the international scope of this conference: Argentina, Australia, Austria, Belgium, Canada, China, Colombia, Czech Republic, Estonia, Finland, France, Georgia, Germany, Greece, Hungary, India, Iran, Israel, Italy, Japan, Jordan, Kazakhstan, Lithuania, Mexico, Nigeria, Norway, Peru, Poland, Portugal, Saudi Arabia, Serbia, Singapore, Slovenia, South Africa, Spain, Suriname, Sweden, Switzerland, The Netherlands, Turkey, United Kingdom and United States of America.

Delegates and speakers included plant breeding researchers and plant breeders (both from the public and private sectors), conservation practitioners and researchers from a wide range of PGR institutes (including crop breeding institutes and companies, gene banks, protected areas, botanic gardens, universities and NGOs), PGR and conservation policy-makers and information managers.

“The conference represents a landmark in the science arena of plant genetic resources”



The 140 delegates of the 'Enhanced Genepool Utilization' international conference outside Churchill college, Cambridge (Photo: Nora Capozio, Bioversity International)

Scientific programme

The conference comprised twelve sessions organized within four themes: 1) characterization techniques, 2) conservation strategies, 3) facilitating CWR and LR use, and 4) informatics development. Fifty-nine oral presentations and 56 posters were shared under these themes.

Theme 1: Characterization techniques

Traditionally, phenotypic characterization and evaluation of conserved diversity involves field trials where breeders select accessions for inclusion in their breeding programmes. However, this method is resource intensive, with the result that the vast majority of the CWR / LR accessions conserved remain uncharacterized and largely unused. This theme examined novel characterization and evaluation techniques to enhance the exploitation of CWR and LR diversity in crop improvement activities. Under this theme there were three sessions: 1.1 – Pre-breeders' use of exotic germplasm; 1.2 – Breeders' use of exotic germplasm; and 1.3 – Novel germplasm characterization techniques.

Theme 2: Conservation strategies

Theme 2 examined the development of systematic CWR and LR conservation strategies at global, regional and national levels and relevant management interventions that will secure and improve the *in situ* and *ex situ* conservation of CWR and LR diversity as a means of promoting CWR and LR use by breeders. Four sessions were convened under this theme: 2.1 – International CWR conservation; 2.2 – International LR conservation; 2.3 – National CWR conservation; and 2.4 – National LR conservation.

Theme 3: Facilitating CWR and LR use

Theme 3 focused on the use of CWR and LR on-farm, *in situ* and in breeding programmes. It also examined the incentive mechanisms that motivate local communities to provide conservation services. Having an enabling policy environment is essential for facilitating conservation and use of CWR and LR. The theme, through dialogue with the stakeholders, also identified the strengths, weaknesses, opportunities and threats for the increased use of CWR and LR. Three sessions were arranged under this theme: 3.1 – Community-based conservation/use; 3.2 – Improving breeders' access to PGRFA; and 3.3 – PGR conservation/use policy.

Theme 4: Informatics development

In order to ensure effective, efficient conservation and use strategies for CWR and LR on-farm and in the wild, we need to know what diversity exists and where, what important traits are contained in specific populations that are of value for crop improvement, and how these resources are changing over time. This theme examined how data are gathered, organized, described, managed and made available to germplasm users. It also examined the types of data necessary for monitoring changes in diversity and what information technology tools are needed for better characterization and conservation management. This theme comprised two sessions: 4.1– Characterization informatics; and 4.2 – Conservation informatics.

Poster session

Fifty-six posters were presented in each of the 12 conference sessions, reflecting the enormous amount of work being un-



Keynote speaker Dr. Mathias Lorieux, International Centre for Tropical Agriculture (CIAT) (Photo: Nora Capozio, Bioversity International)

dertaken in the conservation and utilization of CWR and LR diversity.

The full conference programme and book of abstracts can be consulted online at: www.pgrsecure.org/conference.

Opening session

The conference opening session, led by Ehsan Dulloo, Leader of the Conservation and Availability Programme at Bioversity International, featured opening addresses from guests Tina Barsby (Chief Executive Officer, NIAB), Stephen Weise (Deputy Director General, Research, Bioversity International) and Beat Boller (President, EUCARPIA). Tina Barsby talked about the NIAB mission and objectives and the importance of this event to support plant breeding and food security. Stephan Weise introduced Bioversity's vision that agricultural biodiversity nourishes people and sustains the planet and its mission to deliver scientific evidence, management practices and policy options to use and safeguard agricultural biodiversity to attain sustainable global food and nutrition security. Beat Boller addressed participants by welcoming this joint conference and explaining how important the conference was in highlighting the value of these vital resources, particularly for the plant breeding sector. He also announced the EUCARPIA General Congress to be held in Switzerland and the invitation to participate was marked with a Swiss note with the playing of the alphorn, a traditional Swiss instrument.

A guest lecture was given by Jean Christophe Glaszmann, Director of AGAP (Amélioration Génétique et Adaptation des Plantes Méditerranéennes et Tropicales), a major joint research unit with over 160 scientists from CIRAD, INRA and Montpellier SupAgro, focused on plant genetic improvement and crop adaptation for over twenty tropical and Mediterranean species. Dr. Glaszmann delivered an inspiring lecture entitled 'Making use of germplasm diversity for crop improvement in a range of tropical crops' in which he described the confluences between genome, diversity, actors and populations (click [here](#) to view his presentation).



Ursula Abplanalp playing the alphorn, a traditional Swiss instrument at the conference opening ceremony
(Photo: Nora Capozio, Bioversity International)

Round table discussion, 'Vision of future European PGRFA conservation/use'

A round table discussion on the future of PGRFA conservation and use in Europe was convened in the final conference session. Chaired by Nigel Maxted (PGR Secure Project Coordinator, University of Birmingham), the panel comprised: Eva Thörn (Swedish University of Agricultural Sciences and Chair, EUCARPIA Genetic Resource section); Beat Boller (President, EUCARPIA); Brian Ford-Lloyd (Emeritus Professor, University of Birmingham); Jean-Louis Pham (Senior Programme Officer, Agropolis Fondation) and Lorenzo Maggioni (Coordinator, European Cooperative Programme for Plant Genetic Resources – ECPGR).

Nigel Maxted opened the discussion by reflecting on the significant changes in the organization and operation of PGR research and development in recent years. He invited panelists and delegates to use this forum as an opportunity for an open discussion and expression of individuals' visions for the next ten years. Brian Ford-Lloyd, highlighting the current capability for whole genome sequencing, set the challenge to the CWR community to re-sequence 200 ecogeographically diverse samples of every priority CWR. This challenge was based on examples given during the conference such as rice, barley and alfalfa, which have all been successfully sequenced. Eva Thörn emphasized that the use of genetic resources is a prerequisite for conservation and not the other way round. She also stressed the need to further establish networks on plant breeding and pre-breeding, including intensifying public-private

“Brian Ford-Lloyd set the challenge to the CWR community to re-sequence 200 ecogeographically diverse samples of every priority CWR”

partnerships, especially considering that countries are not self-sufficient in genetic resources. She urged the private sector to be included in public research to find ways to utilize the high technology available today. She finished with a call for more collaboration in different kinds of contexts, regionally, internationally, publicly and privately. Beat Boller outlined his expectations to see more incentives that would lead to a more focused use of PGR in breeding. He underlined the clear need for greater collaborative effort to access materials and to understand their usefulness through information sharing. Jean-Louis Pham said that Europe needs to rethink its policies with regard to PGR, given the challenges for agriculture to produce more food while conserving its resource base. The increasing use of inter- and intra-specific diversity in production systems will require new science, but the traditional science of PGR (collection, conservation, characterization, evaluation and use) remains valid and needs to be supported. He also mentioned the importance of information systems to take account of emerging information technologies and collaborating platforms of citizen science. Lorenzo Maggioni reflected on the long- and medium-term goals of the ECPGR network, which aims to effectively conserve PGRFA *in situ* and *ex situ* and to increase its utilization. Currently the network has decided to work in five main areas: i) establishment of a European gene bank integrated system ('Aegis'); ii) documentation; iii) strengthening relationships with users; iv) *in situ* and on-farm conservation; and v) strengthening the role of the EU in the conservation of genetic resources.



Sónia Dias, Bioversity International (Photo: Nora Capozio, Bioversity International)

“The need for wider collaboration became obvious and the ultimate recommendation was for a continuing, facilitated and inclusive dialogue between the stakeholders”

Several key issues were highlighted during the session which translated into a number of recommendations, including the need for:

- A more strategic approach to the conservation of plant genetic diversity, including greater collaboration between *ex situ* and *in situ* conservation professionals and improved links between the conservation and user communities, including farmers;
- Increased financial support for CWR and LR characterization and conservation which will require all stakeholders to develop a common strategy and to lobby for support from all sectors;
- Alternative solutions for transferring traits from CWR into crops, including the use of re-sequencing and functional analysis;
- Improved exchanges between stakeholders in different parts of the world, for example, by expanding concepts and strategies on how to conserve, access and use CWR and LR diversity developed in Europe to regions such as Africa and Asia;
- Consideration of the impact that legislation is likely to have on *in situ* CWR conservation;
- The development of a plant germplasm release system to store pre-breeding material and a journal to accept and publish this information;
- Improved communication on the importance of PGRFA, bringing together all stakeholders with one voice;
- Greater recognition of farmers as custodians of CWR and LR.

The discussion highlighted viewpoints, expectations and outlooks, bottlenecks, coordination needs, the roles of European institutions, networks' responsibilities, and actions needed by stakeholders. The need for wider collaboration became obvious and the ultimate recommendation was for a continuing,

facilitated and inclusive dialogue between the stakeholders to address the points raised at this conference.

Special events

Delegates had the opportunity to visit the John Innes Centre (JIC), where a tour and lectures took place, thanks to Mike Ambrose, Chris Ridout, Brande Wulff and Adrian Turner. JIC is an independent, international centre of excellence in plant science and microbiology and home to the Germplasm Resources Unit (GRU)—a UK national capability supported by the BBSRC (www.jic.ac.uk/germplasm/). Delegates visited the GRU seed store and exhibits such as *Aegilops* and *Pisum* diversity and wheat evolution. They learnt how CWR and LR are being utilized in a range of JIC research programmes.

Tours of NIAB Innovation Farm's demonstration plots and glasshouses were organized, including CWR and LR diversity exhibits of the PGR Secure project partners (see page 9).

During the conference, delegates enjoyed a traditional English dinner in the historical 16th century Hall of St. John's College, a world food party at which a broad range of PGR from more than 30 countries were sampled, and time exploring the city of Cambridge with its old college buildings and grounds, river walks and punting tours. A post-conference visit to Minsmere National Nature Reserve in Suffolk was enjoyed by many delegates, which concluded with traditional fish and chips on the beach.

Acknowledgements

The conference was supported with funds and in-kind contributions from the EU Seventh Framework Programme, Bioversity International, NIAB Innovation Farm, EUCARPIA, Limagrain and Graminor.



Formal conference dinner in the 16th century Hall of St. John's College
(Photo: Nora Capozio, Bioversity International)

PGR Secure exhibits crop wild relatives and landraces at NIAB Innovation Farm

S. Kell¹, L. Frese², M. Heinonen³, N. Maxted¹, V. Negri⁴, A. Palmé⁵, L. Smith⁶, S.Ø. Solberg⁵ and B. Vosman⁷

¹ School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK. Email: s.kell@bham.ac.uk

² Julius Kühn-Institut, Federal Research Centre for Cultivated Plants (JKI), Institute for Breeding Research on Agricultural Crops, Erwin-Baur-Str. 27, D-06484 Quedlinburg, Germany. Email: lothar.frese@jki.bund.de

³ MTT Agrifood Research Finland, ET-building, FI-31600 Jokioinen, Finland. Email: maarit.heinonen@mtt.fi

⁴ Dipartimento di Scienze Agrarie, Alimentari e Ambientali, University of Perugia, Borgo XX Giugno 74, 06121 Perugia, Italy. Email: valeria.negri@unipg.it

⁵ NIAB Innovation Farm, NIAB, Huntingdon Road, Cambridge, CB3 0LE, UK. Email: lydia.smith@niab.com

⁶ Nordic Genetic Resource Centre, Smedjevägen 3, SE23053 Alnarp, Sweden. Email: anna.palme@nordgen.org; svein.solberg@nordgen.org

⁷ Wageningen UR Plant Breeding, PO Box 16, 6700 AA, Wageningen, The Netherlands. Email: ben.vosman@wur.nl

NIAB Innovation Farm was host institute and sponsor of the joint PGR Secure/EUCARPIA conference, 'Enhanced genepool utilization – capturing wild relative and landrace diversity for crop improvement' convened in Cambridge, UK, 16–20 June 2014. NIAB has a particular strength in practical translation of research to products and Innovation Farm forms the user interface between growers, industry and the research community by working to improve knowledge exchange and to facilitate practical and profitable relationships in order to harness the full potential of plant genetic innovations. One of NIAB Innovation Farm's main facilities is two hectares of land devoted to exhibiting plant genetic resources in field plots and in glasshouses adjacent to a visitor centre containing seminar and networking facilities. The PGR Secure consortium took advantage of this opportunity to display CWR and landrace material to raise awareness of the value of these plant genetic resources for food and agriculture (PGRFA) and to provide a means of attracting users of the material. Summaries of the exhibits are provided below along with links to published information sheets in which readers can find more information.

At least one of the exhibits has gained significant interest from the user community. A group of UK onion and shallot growers who are involved in onion breeding and introducing new lines, showed great interest in the very robust potato onions from Finland and a supplier of alliums to UK supermarkets enquired about them as an opportunity to supply something different for the onion market. MTT Agrifood Research Finland is currently planning how to introduce potato onions in the near future as a niche product for international consumers. The challenge is that at present they are primarily grown for home consumption in Finland and as landraces they are adapted to northern and even local growing conditions.



Lydia Smith (NIAB Innovation Farm) gave guided tours of the exhibits. Here she is explaining the beet exhibit to Adam Drucker (Bioversity International) (Photo: Shelagh Kell)

Beet (mediated by the Julius Kühn-Institut, Germany)

Sugar beet belongs to the genus *Beta* and is one of the few cash crops of worldwide economic importance originating from Europe. The crop is grown within the EU by approximately 150,000 farmers over 1.586 M ha and processed by some 106 factories. Production amounted to 17 MT in 2012/13. Closely related crops are fodder, garden and leaf beet. Wild beet species are increasingly used to feed novel traits into the beet breeding pool, such as resistance to Beet Necrotic Yellow Vein Virus (BNYVV), *Rhizoctonia* root rot and *Cercospora beticola*—the latter which causes leaf spot disease. The exhibit included wild, landrace and old varieties of beet, as well as the modern BNYVV-resistant hybrid sugar beet variety 'Haydn' bred with the resistance trait from *Beta vulgaris* subsp. *maritima*.

For further information, see the [sugar beet information sheet](#) or email lothar.frese@jki.bund.de.

Brassicas (Wageningen UR Plant Breeding and Centre for Genetic Resources, The Netherlands)

The genus *Brassica* contains many important vegetables such as cabbage, broccoli, cauliflower, Brussels sprout and kale, which belong to the species *B. oleracea*, and Chinese cabbage, paksoi and turnip which belong to *B. rapa*. In Europe, brassicas are cultivated across c. 430,000

ha, of which more than half is grown in the East. Brassica vegetables suffer from insect pests, including cabbage thrips, root fly, aphids, caterpillars and whiteflies. The latter are particularly prevalent in Brussels sprout, Savoy cabbage and kale crops. Resistance to these pests is found in wild relatives and landraces of the brassica crops. The exhibited material included amongst others: landraces and varieties of heading cabbage, broccoli and cauliflower, as well as some wild relatives, including *B. villosa*, *B. incana*, *B. fruticulosa* and *B. cretica*.



Greenhouse exhibit displaying *Brassica* wild relatives
(Photo: Ben Vosman)

For further information, see the [brassicac information sheet](#) or email ben.vosman@wur.nl.

Hulless barley (MTT Agrifood Research, Finland)

The hulless barley 'Jorma' is the only hulless barley still in cultivation in Finland where it has been cultivated by one Finnish organic farmer for over 50 years. Although 'Jorma' was released as a commercial variety in the 1970s, >400 year old seed samples studied are the same type as 'Jorma' so we know that this particular hulless barley originates from at least the 17th century. Today, 'Jorma' barley is registered as a landrace under EU regulations, allowing seed production. The amount of flour and other milling output are significantly higher than hulled barley, and according to dietary mineral and protein analysis, 'Jorma' contains more protein, starch and beta-glucan—nutritional qualities that have balancing effects on cholesterol and blood sugar.

For further information, see the [hulless barley information sheet](#) or email maarit.heinonen@mtt.fi.



Close-up of a potato onion in the NIAB Innovation Farm field exhibit
(Photo: Maarit Heinonen)

Forages from the Nordic countries (NordGen – The Nordic Genetic Resource Centre)

Forage crop cultivation is important in the Nordic countries (Norway, Sweden, Finland, Iceland and Denmark) and takes up a large part of the agricultural land. Forages are a central source of fodder and form the basis of milk and meat production. Landraces and wild material have been used in the past to create and improve cultivated varieties and have great potential to do so in the future. They are well adapted to local conditions and are potential sources of traits such as disease resistance, and cold and water-logging tolerance—traits that may be essential for future adaptation to our changing climate. The exhibited material included the wild species 'alpine timothy' (*Phleum alpinum*) which is a close relative of the most commonly cultivated forage species in the north, 'timothy' (*P. pratense*). In addition, the material included two Finnish landraces, one of timothy and one of red clover (*Trifolium pratense*).

For further information, see the [Nordic forages information sheet](#) or email anna.palme@nordgen.org.



Potato onion display among the NIAB Innovation Farm field plots
(Photo: Lydia Smith)

Potato onions (MTT Agrifood Research, Finland)

Potato onion (*Allium cepa* Aggregatum group) is closely related to shallot (Ascalonicum group), although producing larger bulbs and a stronger aroma. It is a northern onion type, although the crop supposedly originated in the East. Potato onions were commonly cultivated in Finland until the mid 20th century when cultivation declined. The remaining crops are all landraces, since there has been no breeding programme. Potato onions are also grown in Sweden and the Baltic Countries. Finnish potato onions were collected during the 1980s and again in 2012. Twenty-two genetically different accessions of potato onions are in long term storage in the field collection at MTT Agrifood Research Finland. Morphologically rich variation amongst accessions was noticed in size (from one to several centimetres in diameter); in shape (from round to oval); in colour of the skin (from light yellow to light red); in resistance to virus and other diseases; in division of bulbs; and in storage qualities.

For further information, see the [potato onion information sheet](#) or email maarit.heinonen@mtt.fi.

Faba bean (University of Perugia, Italy)

Vicia faba originated in the Mediterranean–West Asia region during the Neolithic period and is currently cultivated in many temperate regions for both human and animal consumption. Like other grain legumes it contributes to sustainable agriculture by fixing atmospheric nitrogen and in the past played an important role in the agricultural systems of the Mediterranean Basin. Broad bean (*V. faba* var. *major*) is mostly grown as a grain vegetable because of its large seed size, while the horse bean (*V. faba* var. *equina*) and tick bean (*V. faba* var. *minor*) are grown primarily for animal feed or as a green manure crop. In Europe, the latter two varieties are referred to as field beans. More than 90 cultivars of *V. faba* are registered in the European Community Catalogue, half of which are Dutch, 18 British and 15 Italian. Most of the Italian varieties were registered before 1990. Three Italian landraces were exhibited: one each of broad bean, horse bean and tick bean. The horse bean landrace was from the Amerino district (Umbria Region, central Italy) and is called 'Fava Cottora' (cottora = that cooks well) because it has a short cooking time. This variety recently became a Slow Food Presidium.

For further information email valeria.negri@unipg.it.

Wild and semi-wild vegetables (NordGen – The Nordic Genetic Resource Centre)

The exhibit included accessions of caraway, salsify, angelica and wild carrot.

Caraway

Caraway (*Carum carvi*) is used primarily as a spice in bread, cheese and aquavit. The species grows in the wild, but could also be semi-wild naturalized populations from earlier cultiva-

tions. For example, the introduction of caraway to Iceland is known to have occurred in the mid-17th century by Gísli Magnússon (1621–1696) who allegedly brought seeds from Denmark or the Netherlands and settled in Hlíðarendi in the south of the country where caraway can still be found growing in meadows.

Salsify

The salsify (*Tragopogon porrifolius*) exhibited was collected at the medieval fort ruin of Hammershus in Denmark. The species was most likely introduced for cultivation and has survived as a cultural relict population.

Angelica

Angelica grows in the wild in the Nordic and Arctic regions. Terms such as "angelica gardens" appear in Norwegian records from several hundred years ago. A local variety of angelica called 'Vossakvann', which has solid petioles and a sweet taste, is cultivated in the western parts of Norway. The history of the variety is unknown, but most likely 'Vossakvann' is a result of selection during cultivation. NordGen has some seed collections of wild angelica from Greenland and other Arctic places.

Wild carrot

Wild carrot is quite common in the coastal areas of Europe. The Umbellifer Working Group of the European Cooperative Programme for Plant Genetic Resources (ECPGR) has a project on wild carrot that includes studies on resistance to *Alternaria dauci* (carried out by Julius Kühn-Institut, Germany), as well as characterization of gene bank accessions.

For further information, see the [Nordic vegetables information sheet](#) or email svein.solberg@nordgen.org.



Wild angelica growing on the coast of Iceland (Photo: Svein Ø Solberg)

Phenomics and genomics tools for facilitating brassica crop improvement

B. Vosman¹, K. Pelgrom¹, G. Sharma², R. Voorrips¹, C. Broekgaarden¹, J. Pritchard², S. May³, S. Adobor³, M. Castellanos-Uribe³, M. van Kaauwen¹, B. Janssen⁴, W. van Workum⁴ and B. Ford-Lloyd²

¹ Wageningen UR Plant Breeding, Wageningen University and Research Centre, PO Box 16, 6700 AA, Wageningen, The Netherlands. Email: ben.vosman@wur.nl (Corresponding author)

² School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK

³ University of Nottingham, Loughborough, LE12 5RD, UK

⁴ ServiceXS, Plesmanlaan 1d, 2333 BZ Leiden, The Netherlands

With the expected growth of the human population and the public demand for sustainability there is a strong need for more robust crop varieties, which amongst other characteristics are resistant to pests and diseases. Insects are causing major crop losses and current cultivation methods rely heavily on the use of insecticides, which is undesirable. As a result of climate change, problems with insects are expected to increase (Pritchard *et al.*, 2013) and alternative control measures are needed, of which the use of host plant resistance is the most promising.

Brassica crops, in particular Brussels sprouts, kale and Savoy cabbage suffer from a range of pest insects such as the cabbage aphid (*Brevicoryne brassicae*) and whitefly (*Aleyrodes proletella*). These insects feed on the underside of leaves where they consume sap from the phloem, leading to disrupted plant growth and chlorosis (leaf bleaching). Additionally, they secrete a sugary substance (honeydew) that serves as a good substrate for moulds. Because the cabbage aphid and whitefly feed on the underside of leaves they are difficult to control and growers would therefore benefit strongly from resistant varieties. In the PGR Secure project we aimed to: 1) identify host plant resistance to the cabbage whitefly and cabbage aphid via a germplasm screen, 2) elucidate the resistance mechanism, and 3) provide tools to breeders that will facilitate resistance breeding.

Germplasm screen

As a first step to identify resistant material we screened a collection of 432 accessions, including wild material and landraces of *Brassica oleracea* as well as CWR for whitefly and cabbage aphid resistance in the field (Pelgrom *et al.*, 2012). A selected group of putatively resistant accessions was retested for whitefly resistance in the greenhouse. Resistant accessions were identified among *B. oleracea* var. *capitata* (heading cabbage) landraces and the wild relatives of *B. villosa*, *B. incana* and *B. montana* (Pelgrom *et al.*, 2014). While in heading cabbage resistance is only expressed in plants of at least twelve weeks old, some wild relatives were already starting to express resistance at six weeks. Since farmers plant these crops at an age of 5–6 weeks, this earlier expression of resistance is of great practical importance. Some level of resistance to the cabbage aphid was observed in *B. fruticulosa* and in *B. villosa*.

Location of resistance factors

Plants can defend themselves against phloem-feeding insects by means of physical and chemical barriers. Resistance components can be present in the form of morphological adaptations, such as trichomes (leaf hairs) or wax layers on the surface of the leaf, but may also be present in deeper cell layers or in the phloem. The electrical penetration graph (EPG) technique, in which a phloem-feeding insect is made part of a low voltage electrical circuit, can shed light on the location of the

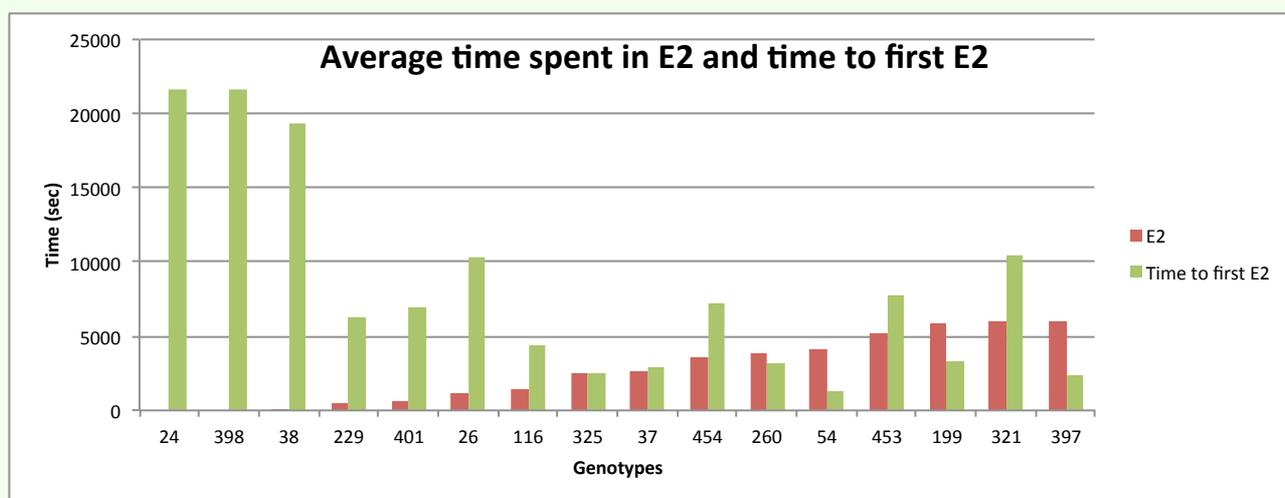


Figure 1 Electrical penetration graph analysis of aphids (*Brevicoryne brassicae*) feeding on different accessions of *Brassica* spp. The average time to reach the phloem phase E2 is shown in green and the time that the aphid is feeding from the phloem is shown in red. Numbers indicate different accessions used (24, 26: *B. incana*; 37, 38: *B. montana*; 397, 398, 401: *B. villosa*; 453, 454: *B. fruticulosa*. All others are *B. oleracea*).

resistance factors in the plant (Tjallingii, 1978). From the EPG readings, information can be obtained about the insect's feeding activities on the plants, which can be translated into parameters that provide information on the presence and location of resistance factors (Tjallingii, 1978; Alvarez *et al.*, 2006). Parameters that can be used are, for instance, the time an insect needs to reach a sustained phloem sap ingestion or the length of the period during which it takes up the phloem sap. Using the EPG technique we could show large differences in feeding behaviour of cabbage aphids on different *Brassica* accessions (Fig. 1). During the six hour recording period, aphids did not reach a period of sustained phloem sap ingestion on some accessions of *B. villosa*, *B. incana* and *B. montana*, whereas they had no problems reaching this stage on some *B. oleracea* accessions. When comparing the activities of the cabbage whitefly on a resistant and susceptible heading cabbage accession it was found that the resistance factors were present at the phloem level (Broekgaarden *et al.*, 2012).

Metabolomics analysis

Secondary metabolites can play an important role in the defence against herbivores. Well described defence compounds in *Brassicaceae* are the glucosinolates (Hopkins *et al.*, 2009). To identify metabolites possibly involved in the resistance against whitefly we performed metabolomics analysis on two sets of plant material. The first set was selected from 125 landrace accessions of heading cabbages differing in levels of whitefly resistance. The second set was selected from an F2 population derived from a cross between two *B. oleracea* cultivars (heading cabbages) (i.e., susceptible 'Christmas Drumhead' and resistant 'Rivera'). Differences in metabolite profiles between resistant and susceptible plants may be used to identify the compounds that are linked to the resistance against cabbage whitefly. From both sets of plant material (landrace accessions and the F2 population) we used the most resistant and most susceptible plants/accessions for analysis. Two complementary metabolomics platforms were used to identify compounds related to susceptibility and resistance—Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS)—in both negative and positive ionization modes. Both the GCMS and LCMS data showed no significant differences in metabolites between the resistant and susceptible groups. Based on this result it is unlikely that the resistance is based on a metabolite. Other mechanisms which may be based on a protein are more likely, although it cannot be excluded that a metabolite not detected by any of the platforms used is the causal agent.

Transcriptomics analysis

We studied differential gene expression in different landraces and species of *Brassica* to obtain information about candidate genes underpinning resistance factors. Genome wide gene expression in response to aphid and whitefly infestation in *Brassica* CWR and landraces was studied using the Affymetrix Arabidopsis Gene 1.0 ST array (Affymetrix, Inc), which consists of 600,941 probes representing 28,500 genes. This array is derived from the inbred *Arabidopsis thaliana* accession 'Columbia'. The TAIR 10 genome annotation, containing 33,602 genes and 41,671 gene models was used (www.affymetrix.com/estore/catalog/prod530004/AFFY/Model-and-applied-research-organisms-Gen-1.1-ST-Array-Strips-Technical-document.pdf).

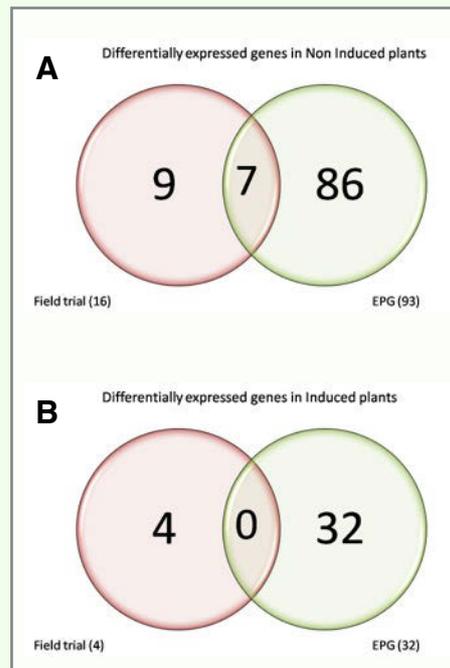


Figure 2 Venn diagram showing the number of differentially expressed genes based on resistance data from field and EPG assessments. Five *Brassica* CWR and 11 *Brassica* landraces were used. The figure shows the total number of differentially expressed genes when resistant and susceptible plants are compared. Panel A shows the number of differentially expressed genes for non-induced plants. Panel B shows the differences in gene expression based on plants induced for 24 hrs with aphids. The total number of differentially expressed genes is given between brackets.

RNA samples were prepared from the leaf tissue of 12 week old plants that were either induced with aphids for 24 hours or non-induced. Differences in gene expression were seen in the sets of material previously classified as resistant or susceptible to aphids based on EPG or field evaluation of resistance (Fig. 2). For non-induced plants, seven differentially expressed genes were common to EPG and field assessment of resistance (out of 16 for field and 93 for EPG). However, for plants induced by aphids there were no common differentially expressed genes, which may be due to the low number of differentially expressed genes observed in the field trial. The differentially expressed genes are considered candidate genes for resistance and are currently being further investigated. However, an important result is also that different genes are revealed as being involved in resistance depending on the way resistance and susceptibility have been assessed (i.e., EPG or field evaluation). However, some are revealed by both methodologies, suggesting the presence of resistance factors at different levels.

Next-generation sequencing and SNP-array development

Molecular markers are an indispensable tool in modern plant breeding. They can be used to make early selection of plant material possible and to facilitate recurrent parent selection, thus speeding up the breeding process. The marker type most widely used is the Single Nucleotide Polymorphisms (SNP). To obtain SNP markers that are informative in *B. oleracea* and its relatives, we carried out a RNAseq analysis of one representative plant of each of the taxa used and on the parent of our mapping populations. In total, the leaf RNA of 15 selected plants was sequenced, resulting in the identification of c. 2 million SNPs. From these SNPs a selection was made based on the position of the SNP on the *B. oleracea* reference genome (Yu *et al.*, 2013). The 90k Affymetrix Axiom array produced contains c. 40,000 SNPs selected from a set of broccoli



Figure 3 Leaves of resistant, hirsute (top) and susceptible, trichome-less (below) plants from the *B. incana* back-cross population

varieties, 21,000 polymorphic SNPs from a set of heading cabbages, 4200 already validated *B. oleracea* SNPs and approximately 5000 SNPs that are polymorphic between *B. oleracea* and the wild relative *B. incana*, as well as 5000 that are polymorphic between *B. oleracea* and *B. montana*. The array also contains c. 5000 SNPs that are polymorphic within *B. fruticulosa*.

Mapping of resistance genes

Host plant resistance to phloem feeding insects can be mediated by several mechanisms (Broekgaarden *et al.*, 2011). QTL mapping was used to identify chromosomal regions involved in whitefly resistance. Based on the germplasm screen, resistant and susceptible plants have been selected as parents for crosses. Within the heading cabbages, an F₂ population was obtained from a cross between the whitefly susceptible cultivar 'Christmas Drumhead' and the resistant 'Rivera'. In this population of 180 plants we measured whitefly adult survival and oviposition rate as well as some morphological characteristics possibly involved in the resistance (time of head formation, leaf wax layer and leaf toughness). The population was genotyped using 150 SNP markers equally spaced over the chromosomes. QTLs were found for the whitefly resistance parameters 'adult survival' and 'oviposition rate', explaining 14% and 13% of the variance, respectively. A strong QTL was found for 'wax layer', explaining 64% of the variance. None of the measured morphological traits co-localized with adult survival and oviposition rate. Although a strong resistance towards the cabbage whitefly was observed in the heading cabbage cultivar 'Rivera', no major QTL was found for survival and oviposition rate. The resistance in this variety is probably based on the interaction of several genes or different resistance mechanisms.

In a second cross we used a fully whitefly resistant plant of the CWR *B. incana* and a susceptible *B. oleracea* cultivar. The resulting F₁ was backcrossed with the *B. incana* parent. In this cross we mapped whitefly resistance to a single locus explaining 57% of the variance for whitefly adult survival and 82% for oviposition rate. At the same locus we also mapped the presence/absence of trichomes (Fig. 3). There was a strong correlation between the presence of trichomes and whitefly adult survival (-0.71) and oviposition rate (-0.89). The presence of the trichomes is likely responsible for the resistance observed.

Work on other populations is still in progress. Information on markers co-segregating with the resistance can be obtained by contacting the corresponding author.

Conclusions

- The PGR Secure project has delivered valuable information on the extent of whitefly and aphid resistance in landrace accessions of *B. oleracea* var. *capitata* as well as in wild relatives of *B. oleracea*.
- The whitefly resistance present in *B. villosa*, *B. incana* and *B. montana* is expressed both in six and twelve week old plants, which indicates that this form of resistance is probably different from that already present in *B. oleracea*. These sources can be used in breeding resistant varieties.
- By combining novel phenomics, genomics and transcriptomics technologies, resistance breeding can be speeded up significantly. The now available 90k Affymetrix Axiom array can play an important role in this.
- The SNP markers linked to the resistance QTLs will facilitate an efficient introgression of the QTLs into high yielding varieties.

Acknowledgements

This research was funded by a grant from the EU 7th framework programme for the PGR Secure project (Grant agreement no: 266394) and a grant from the ministry of Economic affairs of the Netherlands (project nr. BO-26.03-009-001) for the contribution of the Wageningen UR Plant Breeding partners.

References

- Alvarez, A.E., Tjallingii, W.F., Garzo, E., Vleeshouwers, V., Dicke, M. and Vosman, B. (2006) Location of resistance factors in the leaves of potato and wild tuber-bearing *Solanum* species to the aphid *Myzus persicae*. *Entomologia Experimentalis et Applicata* 121: 145–157.
- Broekgaarden, C., Snoeren, T.A.L., Dicke, M. and Vosman, B. (2011) Exploiting natural variation to identify insect-resistance genes. *Plant Biotechnology Journal* 9: 819–825.
- Broekgaarden, C., Riviere, P., Steenhuis, G., del Sol Cuenca, M., Kos, M. and Vosman, B. (2012) Phloem-specific resistance in *Brassica oleracea* against the whitefly *Aleyrodes proletella*. *Entomologia Experimentalis et Applicata* 142: 153–164.
- Hopkins, R.J., van Dam, N.M. and Van Loon, J.J.A. (2009) Role of glucosinolates in insect-plant relationships and multitrophic interactions. *Annual Review of Entomology* 54: 57–83.
- Pelgrom, K., Sharma, G., Broekgaarden, C., Voorrips, R., Bas, N., Pritchard, J., Ford-Lloyd, B. and Vosman, B. (2012) Looking for resistance to phloem feeders in *Brassica oleracea*. *Crop wild relative* 8: 12–14.
- Pelgrom, K.T.B., Broekgaarden, C., Voorrips, R.E., Bas, N., Visser, R.G.F. and Vosman, B. (2014) Host plant resistance towards the cabbage whitefly in *Brassica oleracea* and its wild relatives. *Euphytica*. DOI: 10.1007/s10681-014-1306-y.
- Pritchard, J., Broekgaarden, C. and Vosman, B. (2013) Effects of climate change on plant-insect interactions and prospects for resistance breeding using genetic resources. In: Jackson, M., Ford-Lloyd, B. and Parry, M. (eds), *Plant Genetic Resources and Climate Change*. CABI Publishing, Wallingford. Pp. 270–284.
- Tjallingii, W.F. (1978) Electronic recording of penetration behaviour by aphids. *Entomologia Experimentalis et Applicata* 24: 721–730.
- Yu, J., Zhao, M., Wang, X., Tong, C., Huang, S., Tehrim, S., Liu, Y., Hua, W. and Liu, S. (2013) Bolbase: a comprehensive genomics database for *Brassica oleracea*. *BMC Genomics* 14: 664.

Successful use of crop wild relatives in breeding: easier said than done

K. Pelgrom, C. Broekgaarden, R. Voorrips and B. Vosman

Wageningen UR Plant Breeding, Wageningen University and Research Centre, PO Box 16, 6700 AA Wageningen, The Netherlands. Email: ben.vosman@wur.nl

With a growing population and improving diets there is a need to double our food supply by 2050. Agriculture will not only face the increasing demand for food but also the threat of climate change. These problems bring great responsibilities but also opportunities for the breeding community. The use of CWR genes could play an important role in improving crop performances that will allow us to feed the world in the near future. Breeding companies are well aware of the potential use of CWR genes for breeding programmes; they increasingly build-up their own genetic resources and participate in collection expeditions performed by national gene banks. These CWR accessions could possess important traits not only for abiotic stresses, like drought and salt tolerance, but also against pests and diseases.

Within the PGR Secure project we took a closer look at biotic stress resistance tolerance in brassica crops with emphasis on phloem feeding insects. From literature it is known that the CWR *Brassica fruticulosa* confers resistance to many insects including the cabbage aphid (*Brevicoryne brassicae*), cabbage rootfly (*Delia radicum*) and cabbage whitefly (*Aleyrodes proletella*) (Ramsey and Ellis, 1996; Ellis *et al.*, 1999; Jensen *et al.*, 2002; Pink *et al.*, 2003; Kumar *et al.*, 2011). To transfer the genes that play a role in the resistance, interspecific crosses between *B. oleracea* and *B. fruticulosa* are needed. To this purpose, a cabbage aphid susceptible *B. oleracea* landrace was hand pollinated with *B. fruticulosa* pollen. Most attempts led to flower abortion or poor seed set. Fortunately one seed germinated and phenotypic characteristics from both parents could be observed in the F1 plant (Fig. 1). Leaf samples from the F1 plant and parental accessions were collected and an-



Figure 1 F1 Hybrid with lobed leaf shape (*B. fruticulosa*) and relatively large leaf size (*B. oleracea*). During plant development a wax layer is formed which is typical for *B. oleracea* but absent in *B. fruticulosa* plants.

“Agriculture will not only face the increasing demand for food but also the threat of climate change”

alysed by flow cytometry to determine the amount of DNA in the nuclei of the plant cells and DNA was isolated to identify genetic variation by single nucleotide polymorphisms (SNPs). The results from both methods indicated that we were dealing with a real F1 hybrid. To our knowledge this is the first report that mentions a successful (hand pollinated) interspecific cross between *B. oleracea* and *B. fruticulosa*. The first F1 flowers appeared 14 weeks after sowing (Fig. 2), which is quite different from the parents; *B. oleracea* is biennial and needs vernalization to flower, while *B. fruticulosa* is annual and flowers four weeks after sowing. Pollen of the F1 hybrid were further analysed for their viability but no pollen tubes could be detected. Nevertheless, selfings and backcrosses (pollinated with the susceptible *B. oleracea* landrace) were made. The first results looked promising and led to thickening of the pistil but never resulted in successful seed set (Fig. 3). So far, ovule rescue did not succeed in producing viable plant material.

In many cases, biological barriers like hybrid sterility (as we observed in our research), still prevent the successful use of wild material. Even though ovule and embryo rescue can in some cases be used to overcome these barriers and increase



Figure 2 The F1 formed flowers have relatively large pistils and small stamens without viable pollen. Parental accessions differ in chromosome number: *B. oleracea* $2n=18$, *B. fruticulosa* $2n=16$.



Figure 3 Thickening of F1 pistils from the backcross with *B. oleracea*. Pistils shrivelled in time without successful seed set.

the chance of survival and production of viable plant material for further research (Bang and Kaneko, 2014) this approach often fails to produce the desired results. Even where crossing barriers can be overcome, retention of undesirable agronomic traits remains a prominent technical limitation in the use of CWR. Crosses usually produce plants that have poor agronomic performance, and often undesirable traits (Hajjar and Hodgkin, 2007). However, this last hurdle can be tackled in an efficient way as molecular markers are available in large numbers (Vosman *et al.*, 2014), and can be used to efficiently remove linkage drag. These novel techniques will likely contribute to an increasing number of cultivars containing CWR genes. Therefore, effective CWR conservation *in situ* and *ex situ* will be of great importance as well as the availability of the material for pre-breeding purposes.

References

- Bang, S.W. and Kaneko, Y. (2014) Interspecific and intergeneric hybridization and chromosomal engineering of Brassicaceae crops. *Breeding Science* 64: 14–22.
- Ellis, P.R., Pink, D.A.C., Barber, N.E. and Mead, A. (1999) Identification of high levels of resistance to cabbage root fly, *Delia radicum*, in wild Brassica species. *Euphytica* 110(3): 207–214.
- Hajjar, R. and Hodgkin, T. (2007) The use of wild relatives in crop improvement: a survey of developments over the last 20 years. *Euphytica* 156(1–2): 1–13. DOI:10.1007/s10681-007-9363-0.
- Jensen, E.B., Felkl, G., Kristiansen, K. and Andersen, S.B. (2002) Resistance to the cabbage root fly, *Delia radicum*, within *Brassica fruticulosa*. *Euphytica* 124(3): 379–386.
- Kumar, S., Atri, C., Sangha, M.K. and Banga, S.S. (2011) Screening of wild crucifers for resistance to mustard aphid, *Lipaphis erysimi* (Kaltenbach) and attempt at introgression of resistance gene(s) from *Brassica fruticulosa* to *Brassica juncea*. *Euphytica* 179(3): 461–470.
- Pink, D.A.C., Kift, N.B., Ellis, P.R., McClement, S.J., Lynn, J. and Tatchell, G.M. (2003) Genetic control of resistance to the aphid *Brevicoryne brassicae* in the wild species *Brassica fruticulosa*. *Plant Breeding* 122(1): 24–29.
- Ramsey, A.D. and Ellis, P.R. (1996) Resistance in wild brassicas to the cabbage whitefly, *Aleyrodes proletella*. *Acta Horticulturae* 407: 507–514.
- Vosman, B., Pelgrom, K., Sharma, G., Voorrips, R., Broekgaarden, C., Pritchard, J., May, S., Adobor, S., Castellanos-Uribe, M., van Kaauwen, M., Janssen, B., van Workum, W. and Ford-Lloyd, B. (2014) Phenomics and genomics tools for facilitating brassica crop improvement. *Crop wild relative* 10: 12–14.

Two predictive characterization approaches to search for target traits in crop wild relatives and landraces

I. Thormann¹, M. Parra Quijano², J.M. Iriondo³, M.L. Rubio Teso³, D.T. Endresen⁴, N. Maxted⁵, S. Dias¹ and M.E. Dooloo¹

¹ Bioversity International, Italy

² International Treaty on Plant Genetic Resources for Food and Agriculture – FAO, Italy

³ Universidad Rey Juan Carlos, Spain

⁴ Natural History Museum, University of Oslo, Norway

⁵ University of Birmingham, UK

Characterization and evaluation (C&E) of CWR and landraces (LR)—essential for enhancing their conservation and use—has nearly always involved an element of prediction. In practice, breeders rarely choose accessions for field characterization and evaluation randomly—where possible they select accessions they believe are likely to contain the desired traits. Advances in molecular and GIS analysis techniques mean that predictions of which accessions are likely to contain desired traits are now significantly more objective (evidence-based) than they were previously. Collectively, such predictive characterization approaches involve GIS analysis together with environmental and agro-ecological data

and they present a more cost effective approach than subjective traditional phenotypic characterization methods.

Predictive characterization methods are predictive in the sense that they assign the potential of trait presence to uncharacterized germplasm (either *ex situ* or *in situ*) using i) matching of (a)biotic characteristics associated with a particular location, ii) environmental information associated with a particular location, and iii) previously recorded C&E data of trait occurrence associated with a particular location. In each case a predictor is used to build a hypothesis that germplasm from a particular location will be genetically differentiated. One of the first

Box 1 Examples of predictive association studies and identification of pest and pathogen resistant and drought tolerant material through the use of FIGS

- Predictive association between trait data and ecogeographic data for Nordic barley landraces (Endresen, 2010)
- Predictive association between biotic stress traits and ecogeographic data for wheat and barley (Endresen *et al.*, 2011)
- Wheat stem rust resistance linked to environmental variables (Bari *et al.*, 2012)
- Resistance to stem rust (*Ug99*) in bread wheat and durum wheat (Endresen *et al.*, 2012)
- Sources of wheat resistance to Sunn pest, *Eurygaster integriceps* (El Bouhssini *et al.*, 2009)
- Powdery mildew resistance in wheat (Kaur *et al.*, 2008; Bhullar *et al.*, 2010)
- Sources of resistance in bread wheat to Russian wheat aphid, *Diuraphis noxia* (El Bouhssini *et al.*, 2011)
- Traits related to drought adaptation in *Vicia faba* genetic resources identified (Khazaei *et al.*, 2013)

systematic applications of finding a predictive link between a resistance trait and a set of environmental parameters was named the Focused Identification of Germplasm Strategy (FIGS) (Mackay and Street, 2004; Street *et al.*, 2008), developed at the International Centre for Agricultural Research in the Dry Areas (ICARDA). FIGS methods have mainly been applied to major crops, in particular wheat and barley (see Box 1 for examples).

Building upon the foundation of the FIGS approach, studies that use ecogeographical information or previously recorded C&E data have been developed and were tested within the context of the PGR Secure project for their applicability to CWR and LR of the project's target genera *Avena*, *Beta*, *Brassica* and *Medicago*. Two methods were explored: the ecogeographical filtering method and the calibration method. These methods, using an agro-ecological approach, were used to search for populations and accessions with targeted adaptive traits.

Ecogeographical filtering method

The ecogeographical filtering method combines the spatial distribution of the target taxon on an ecogeographical land characterization (ELC) map (Parra Quijano *et al.*, 2012a, b) with the identification of environmental profiles that are likely to impose selection pressure for the adaptive trait investigated, to filter occurrence records.

The ELC map is based on the variables most relevant for adaptation and for determining the species' distribution, and aims at representing the adaptive scenarios that are present over the territory studied. Figure 1 shows the European ELC map for *Avena* and Table 1 provides the variables that were used to develop the map. As a first step in this method the ecogeographical categories from the ELC map are assigned to each occurrence record according to its coordinates and the

Table 1 Variables identified based on literature review and expert knowledge as relevant for the geographical distribution of *Avena* and used for the ELC map development

Bioclimatic variables	Edaphic variables	Geophysical variables
BIOCLIM 1	Topsoil Sand Fraction (T_SAND)	Elevation
BIOCLIM 4	Drainage Class	Longitude
BIOCLIM 6	Total Exchangeable Bases in Topsoil (T_TEB)	Latitude
BIOCLIM 11	Topsoil Salinity (T_ECE)	Northness
BIOCLIM 19	Topsoil Organic Carbon (T_OC)	Eastness

records are then grouped according to their ELC map category.

After all georeferenced occurrences have been ecogeographically characterized, the second step is to select occurrences from each group which comply with specific environmental requirements related to the traits of interest: the specific ecogeographical variables that best describe and delimit the environmental profile likely to impose selection pressure for the adaptive trait of interest are used for further filtering to obtain a final subset of occurrences. Table 2 provides the variables used for aluminium toxicity in *Avena*.

Calibration method

The calibration method uses existing characterization and evaluation data for the trait of interest together with ecogeographical data specific to the environment at collecting sites from where these accessions were collected to identify existing relationships between the trait and the environment. Based on these relationships it calibrates a prediction model. This prediction model is then applied to other non-evaluated accessions to identify those that, according to this model, are likely to have a higher probability of genetic adaptation for a target trait property. The model therefore aims to identify a subset that is more likely to show the target trait property than a subset merely selected randomly (see e.g. Endresen *et al.*, 2011, 2012; Bari *et al.*, 2012).

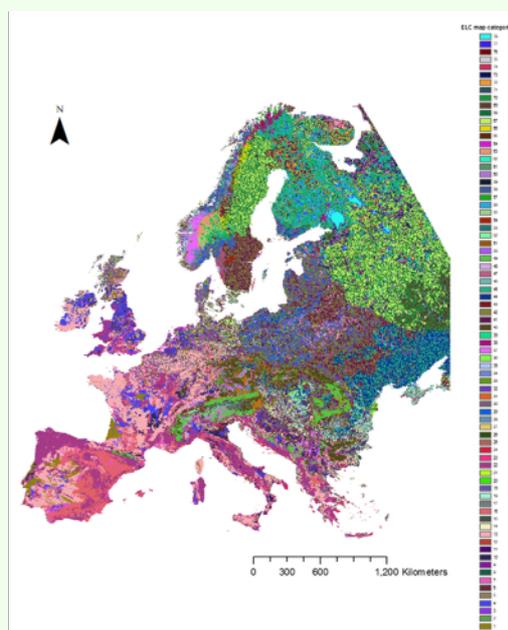


Figure 1 ELC map for *Avena* created in the PGR Secure project

Table 2 Variables and variable thresholds used to delimit environments that potentially are related to aluminium toxicity

Genus	Trait	Variables	Threshold values
Avena	Aluminium toxicity	Soil pH; Soil organic carbon content T_OC	<pH 5.5; <1,2% T_OC

Results

When applied to a dataset containing all georeferenced records for European LR and CWR of the four target genera of the PGR Secure project, the ecogeographical filtering method generated subsets of LR and CWR occurrences that are assumed to have a potentially higher likelihood of containing genetic diversity relevant for the following target tolerance/resistance traits: aluminium toxicity for *Avena*, drought for *Beta*, drought and salinity for *Brassica*, and frost for *Medicago*. The evaluation data available for LR and CWR of these four genera was insufficient to apply the calibration method.

The testing and implementation of these two methods highlighted some critical aspects connected to predictive characterization:

- Accurate georeferenced information for all occurrences is important to allow proper extraction of climate, edaphic and geophysical data.
- Increasing the number and improving the quality of environmental variables that are made available globally will make the methods more accurate.
- ELC maps and calibration models need to correctly reflect the assumption that is implemented in these methods (i.e. that different environmental conditions generate different selective pressures and genetic differentiation of adaptive value).
- The environmental profiles that promote target traits in landraces or CWR need to be carefully described with environmental variables for which we have data in the territory.
- These methods are not appropriate for modern cultivars as they are not expected to show this association between traits and the environment. This is because their traits have not arisen as a result of natural selection but have been artificially selected to provide a high yield under a wide range of environmental conditions.
- The ecogeographical filtering method is the method better suited for CWR, as it is very unlikely that a sufficient number of C&E data records for a specific CWR species required to implement the calibration method will be available.

The power of FIGS and other predictive characterization methods is to reduce the size and therefore cost of field trials by reducing the set of candidate accessions which the breeder needs to screen before finding novel alleles for a target trait. This is achieved through the less expensive pre-screening against an environmental profile. These methods therefore help to more efficiently utilize the limited and costly land area and human working time for field screening and increase the capacity for selecting better targeted material for potential use in plant breeding.

References

- Bari, A., Street, K., Mackay, M., Endresen, D.T.F., de Pauw, E. and Amri, A. (2012) Focused identification of germplasm strategy (FIGS) detects wheat stem rust resistance linked to environmental variables. *Genetic Resources and Crop Evolution* 59: 1465–1481. DOI:10.1007/s10722-011-9775-5.
- Bhullar, N., Mackay, M. and Keller, B. (2010) Genetic diversity of the *Pm3* powdery mildew resistance alleles in wheat gene bank accessions as assessed by molecular markers. *Diversity* 2: 768–786.
- El Bouhssini, M., Street, K., Joubi, A., Ibrahim, Z. and Rihawi, F. (2009) Sources of wheat resistance to Sunn pest, *Eurygaster integriceps* Puton, in Syria. *Genetic Resources and Crop Evolution* 56: 1065–1069.
- El Bouhssini, M.E., Street, K., Amri, A., Mackay, M., Ogbonnaya, F.C., Omran, A., Abdalla, O., Baum, M., Dabbous, A. and Rihawi, F. (2011) Sources of resistance in bread wheat to Russian wheat aphid (*Diuraphis noxia*) in Syria identified using the focused identification of germplasm strategy (FIGS). *Plant Breeding* 130: 96–97. DOI:10.1111/j.1439-0523.2010.01814.x.
- Endresen, D.T.F. (2010) Predictive association between trait data and ecogeographic data for Nordic barley landraces. *Crop Science* 50: 2418–2430. DOI: 10.2135/cropsci2010.03.0174.
- Endresen, D.T.F., Street, K., Mackay, M., Bari, A. and De Pauw, E. (2011) Predictive association between biotic stress traits and ecogeographic data for wheat and barley landraces. *Crop Science* 51: 2036–2055. DOI: 10.2135/cropsci2010.12.0717.
- Endresen, D.T.F., Street, K., Mackay, M., Bari, A., De Pauw, E., Nazari, K. and Yahyaoui, A. (2012) Sources of resistance to stem rust (*Ug99*) in bread wheat and durum wheat identified using focused identification of germplasm strategy. *Crop Science* 52(2): 764–773. DOI: 10.2135/cropsci2011.08.0427.
- Kaur, N., Street, K., Mackay, M., Yahiaoui, N. and Keller, B. (2008) Allele mining and sequence diversity at the wheat powdery mildew resistance locus *Pm3*. In: Appels, R., Eastwood, R., Lagudah, E., Langridge, P., Mackay, M., McIntyre, L. and Sharp, P. (eds.), *11th International Wheat Genetics Symposium, Volume 1*. Sydney University Press, Brisbane. Pp. 32–34.
- Khazaei, H., Street, K., Bari, A., Mackay, M. and Stoddard, F.L. (2013) The FIGS (focused identification of germplasm strategy) approach identifies traits related to drought adaptation in *Vicia faba* genetic resources. *PLoS ONE* 8(5): e63107. DOI:10.1371/journal.pone.0063107.
- Mackay, M.C. and Street, K. (2004) Focused identification of germplasm strategy – FIGS. In: Black, C.K., Panozzo, J.F. and Rebetzke, G.J. (eds.), *Cereals 2004. Proceedings of the 54th Australian Cereal Chemistry Conference and the 11th Wheat Breeders' Assembly, 21–24 September 2004, Canberra, Australian Capital Territory (ACT)*. Cereal Chemistry Division, Royal Australian Chemical Institute, Melbourne, Australia. Pp. 138–141.
- Parra Quijano, M., Iriondo, J.M. and Torres, E. (2012a) Ecogeographical land characterization maps as a tool for assessing plant adaptation and their implications in agrobiodiversity studies. *Genetic Resources and Crop Evolution* 59: 205–217.
- Parra Quijano, M., Iriondo, J.M., Frese, L. and Torres, E. (2012b) Spatial and ecogeographic approaches for selecting genetic reserves in Europe. In: Maxted, N., Dulloo, M.E., Ford-Lloyd, B.V., Frese, L., Iriondo, J. and Pinheiro de Carvalho, M.A.A. (eds.), *Agrobiodiversity Conservation: Securing the Diversity of Crop Wild Relatives and Landraces*. CABI Publishing, Wallingford. Pp. 20–28.
- Street, K., Mackay, M., Zuev, E., Kaul, N., El Bouhssini, M., Konopka, J. and Mitrofanova, O. (2008) Diving into the genepool – a rational system to access specific traits from large germplasm collections. In: Appels, R., Eastwood, R., Lagudah, E., Langridge, P., Mackay, M., McIntyre, L. and Sharp, P. (eds.), *The 11th International Wheat Genetics Symposium proceedings*. Sydney University Press, Australia. <http://hdl.handle.net/2123/3390>

Europe's crop wild relative diversity: from conservation planning to conservation action

S. Kell, B. Ford-Lloyd and N. Maxted

School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK. Email: s.kell@bham.ac.uk

Europe is an important centre of diversity of many crops and their wild relatives and these CWR are potential genetic resources for crop improvement and food security. Food crops with significant CWR diversity native to the region include wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.), sugar beet (*Beta vulgaris* L.), cabbage and other brassicas (*Brassica* L. spp. and allied genera), onion and other alliums (*Allium* L. spp.), asparagus (*Asparagus officinalis* L.), lettuce (*Lactuca sativa* L.) and apple (*Malus domestica* L.). Forage and fodder crops with CWR native to Europe include annual meadow grass (*Festuca pratensis*), white clover (*Trifolium repens*), alfalfa (*Medicago sativa* L.) and common vetch (*Vicia sativa* L.). Other crops of socio-economic importance with native wild relatives in the region are forestry species such as *Abies alba* Mill., *Populus nigra* L. and *Quercus ilex* L., ornamentals such as species of *Dianthus* L., *Euphorbia* L., *Geranium* L. and *Primula* L., medicinal and aromatic plants such as species of *Anemone* L., *Campanula* L., *Helianthemum* Mill., *Orchis* L. and *Verbascum* L., as well as a range of herb, spice, environmental, and industrial crops.

Increased global awareness of the value of CWR for crop improvement—particularly due to the projected impact of climate change on crop production and the need for insurance against food insecurity—has highlighted the imperative for

greater targeted CWR conservation and utilization efforts. CWR diversity is an important resource for the maintenance of food security and for safeguarding the substantial economic gains to Europe through crop production in the region. However, despite their recognized value, their conservation has been largely neglected, in part due to the disconnection between the agencies responsible for the conservation of plant genetic resources for food and agriculture (PGRFA) and those responsible for the conservation of wild plant populations in general or the habitats in which they grow.

Recent advances in our understanding of CWR diversity in the region, as well as in planning for its complementary conservation (i.e., both *in situ* and *ex situ*), provides a solid foundation for the development of a strategic approach to their conservation in Europe based on a range of commonly agreed and widely tested scientific concepts and techniques. However, achieving effective conservation and utilization of European CWR diversity as a means to promote food and economic security in the region will require a coherent, regionally coordinated policy and the appropriate resources to fund their conservation, characterization and evaluation. To achieve sustainable conservation of CWR and maximize their sustainable exploitation in Europe, there is an imperative to develop an EU-led policy to harmonize their conservation, characterization and evaluation with existing biodiversity conservation and agricultural initiatives, and to develop new initiatives where necessary.

As reported in *Crop wild relative* Issue 9 (Editorial), a concept for *in situ* conservation of CWR has been prepared (Maxted *et al.*, 2013) to guide EU and national policy development and to act as a blueprint to drive concerted actions throughout the region. The document has undergone a process of review by the ECPGR Steering Committee and members of the Wild Species Conservation in Genetic Reserves Working Group and a revised document prepared (Maxted *et al.*, 2015 – www.pgrsecure.org/documents/Concept_v2.pdf) (Fig. 1). The Concept is based on a comprehensive background document (www.pgrsecure.org/documents/Background_document.pdf) which details the imperative for CWR conservation in Europe, the national, regional and integrated approaches to their conservation, and the requirement for a new policy paradigm to secure their genetic diversity. The document also addresses a number of back-stopping elements, including a) methods of diversity and gap analysis to identify priority populations for conservation action; b) population management inside and outside protected areas; c) a proposal for integrating *in situ* and *ex situ* conservation and the sustainable use of CWR genetic diversity within the ECPGR; d) options to promote integration between PGRFA and nature conservation communities; and e) options to promote awareness of the value of CWR diversity and for raising funding for their conservation in Europe.

Preserving diversity: a concept for *in situ* conservation of crop wild relatives in Europe: version 2



Nigel Maxted, Alvina Avagyan, Lothar Frese, José Iriondo, Joana Magos Brehm, Alon Singer and Shelagh Kell

Figure 1 The concept for *in situ* conservation of CWR in Europe

A strategy for the conservation of Europe's CWR diversity, detailing regional priority taxa and populations is under preparation. Initial results highlight some 200 species that are an immediate priority for conservation planning based on a) their relationship to crops of high economic and food security importance in Europe, and b) their relative threat status. The responsibility for conserving these priority species is Europe-wide with some 30 countries containing native, wild populations of 20 or more species. Initial results of gap analyses reveal that only around half of these priority species occur within protected areas, and alarmingly that less than half are represented in gene bank collections. Further, approximately half of the species found in gene bank collections are represented by only eight accessions or less. The full European strategy document will be published online and results used to inform the development and implementation of an integrated CWR conservation strategy for Europe (Fig. 2).

References

- Maxted, N., Avagyan, A. Frese, L., Iriondo, J.M., Magos Brehm, J., Singer, A. and Kell, S.P. (2013) *Preserving diversity: a concept for in situ conservation of crop wild relatives in Europe*. In Situ and On-farm Conservation Network, European Cooperative Programme for Plant Genetic Resources, Rome, Italy. www.pgrsecure.org/documents/Concept.pdf
- Maxted, N., Avagyan, A. Frese, L., Iriondo, J.M., Magos Brehm, J., Singer, A. and Kell, S.P. (2015) *Preserving diversity: a concept for in situ conservation of crop wild relatives in Europe: Version 2*. Rome, Italy: Wild Species Conservation in Genetic Reserves Working Group, European Cooperative Programme for Plant Genetic Resources. www.pgrsecure.org/documents/Concept_v2.pdf



Aegilops cylindrica, a secondary wild relative of wheat which has been used to confer salt tolerance and has potential for pest resistance (Photo: Pavol Eliáš)

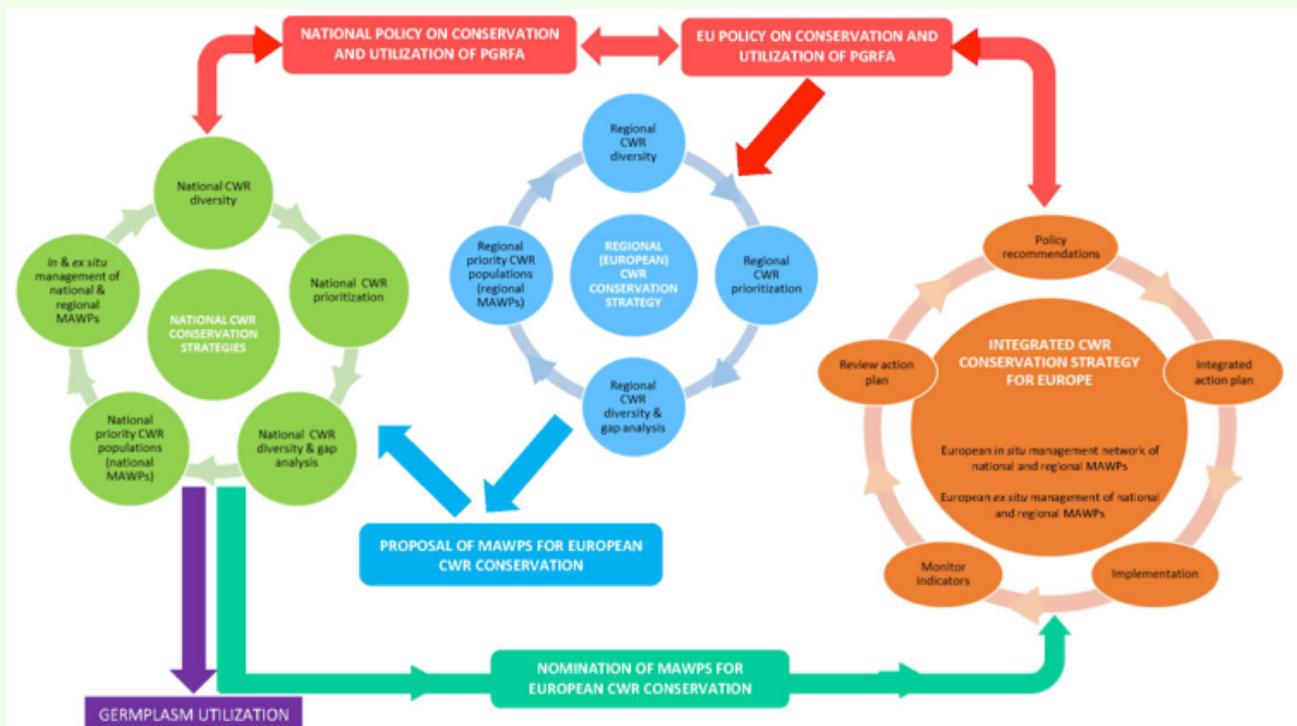


Figure 2 Schematic representation of the concept for *in situ* conservation of CWR in Europe (Maxted *et al.*, 2013, 2015). MAWPs = Most Appropriate Wild Populations – a new paradigm for CWR conservation.

Discovering Finnish crop wild relative diversity and gaps in their conservation

H. Fitzgerald¹ and H. Korpelainen²

¹Botany Unit, Finnish Museum of Natural History, University of Helsinki, Finland. Email: heli.fitzgerald@helsinki.fi

²Department of Agricultural Sciences, University of Helsinki, Finland.

The Finnish CWR conservation strategy was created within the EU-funded PGR Secure project. The compiled CWR checklist for Finland includes 1905 taxa and the prioritized list contains 209 taxa. *In situ* and *ex situ* gap analyses were conducted for the prioritized taxa. Using the complementarity analysis method it was possible to identify key areas for *in situ* conservation to be recommended as possible genetic reserve sites. Gaps in *ex situ* conservation were also identified and suggestions made for the collection of genetically representative samples of CWR populations. The strategy gives recommendations for the future conservation of Finnish CWR diversity and suggestions for its implementation. This article briefly describes the results of the *in situ* and *ex situ* gap analyses.

In situ gap analysis

Through the *in situ* gap analysis it was found that there are no current conservation efforts specifically for CWR species in Finland, but the threatened CWR species are included in the existing conservation programmes. However, the conservation of these species has not had the perspective of them being CWR. Therefore, an analysis was carried out to find the hotspots of CWR distribution in Finland to suggest them as possible locations for CWR genetic reserves in the future. The identification of the key areas containing CWR in Finland was undertaken by a complementarity analysis. The idea was to find out the minimum number of sites with the maximum number of CWR taxa. The *in situ* gap analysis was carried out in ArcGIS using vascular plant distribution data from the Kasviatlas - Suomen putkilokasvien levinneisyyskartasto (Lampinen *et al.*, 2012), Maanmittauslaitos (Maanmittauslaitos, 2013) and OIVA (SYKE, 2012). Nature conservation area data for the Åland region were obtained from the regional government. Through the complementarity analysis, five most CWR species rich areas were found within Finland (Fig. 1). These CWR hotspot sites, if established as genetic reserves, would conserve over 60% of the priority species.

Ex situ gap analysis

The *ex situ* gap analysis was carried out by finding out which of the priority taxa were already sufficiently conserved in *ex situ* collections and which were in need of conservation. In Finland, wild plant species collections are found mostly in botanic gardens, with some additional CWR collections in NordGen (the Nordic Genetic Resource Centre). Also, a seedbank for threatened native species is currently being established in Finland through the EU Life+ funded ESCAPE project, which collects and conserves threatened Finnish vascular plant species.

The gap analysis revealed significant gaps in the *ex situ* conservation of CWR in Finland. It was found that 75% of

the priority CWR are not in *ex situ* collections at all. The rest (25%) are found in *ex situ* collections but they are mostly not genetically representative, since they have been collected from one population only. These species would need additional collecting from diverse locations of their distribution. Only 3% of CWR taxa in *ex situ* collections have been collected from a minimum of five locations. However, many of the threatened CWR taxa will be covered by the present collection programme of threatened Finnish wild species. The biggest gaps in CWR *ex situ* conservation are therefore those threatened taxa which are not included in the present collection lists, and the non-threatened taxa untargeted by any collecting activity. These species should be a priority for *ex situ* collecting of CWR in the future.

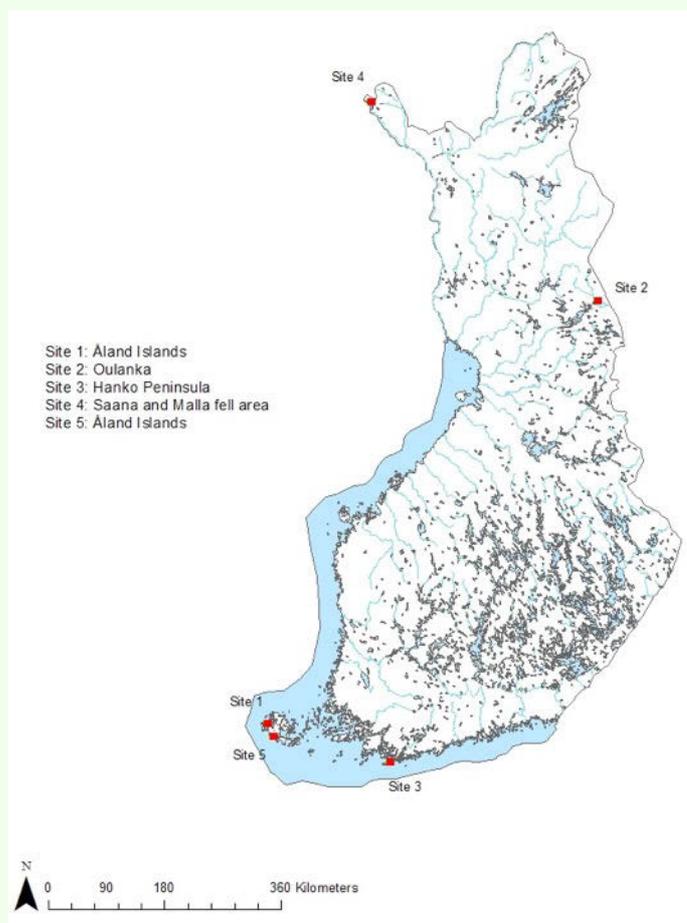


Figure 1 The five crop wild relative hotspots in Finland (Source: Fitzgerald, 2013)



Figure 2 Dry meadow in the Åland Islands (Photo: Jouko Lehmuskallio, Luontoportti)

Overview of the five CWR hotspot sites in Finland

Sites 1 and 5: Åland Islands

The most CWR species rich area in Finland is the Åland Islands, which is an autonomous archipelago province in the Baltic Sea. Half of the priority taxa were found to exist in this region. Åland comprises of the main island and thousands of smaller islands. The nature in Åland is unique and contains some of Finland's most species-rich areas. The Åland islands are covered by calcareous soil, which with the traditional agriculture has led to the development of species-rich wooded, coastal and dry meadows (Fig. 2), groves, open pastures and heaths. The main threat to plant diversity in Åland is overgrowing of meadows due to changes in agricultural practices.

The complementarity analysis found two 10x10 km grids to be particularly CWR species rich. Both of them are on the main island. Site 1 has 65 priority CWR not found in other hotspots. Site 5 has 63 priority taxa, 13 of which are not found in other hotspots. The first site is located in the Eckerö and Hammarland region, and the second one in the Marihamn capital city area and on some smaller islands, such as Nätö. Both Eckerö and Nätö are known to have a high diversity of species in general and there are several small nature conservation areas in both regions. Åland's CWR include several species not found elsewhere in Finland. CWR of a number of priority food and forage crops are found in these two regions, including species of *Fragaria*, *Malus*,

Lathyrus, *Vicia*, *Allium*, *Festuca*, *Phleum*, *Poa*, *Trifolium*, *Ribes* and *Rubus*.

Site 2: Oulanka

The second highest concentration of CWR, when the species found in the first site are removed from the analysis, is located in the Oulanka area. This site includes 24 priority CWR not found in other hotspots and 39 priority taxa in total. A large proportion of the 10x10 km grid is covered by the Oulanka National Park. The Oulanka area in the northern Ostrobothnia region has an interesting combination of northern, southern and eastern species. Many of them are found at the edge of their distribution range. The diverse flora and the occurrence of many threatened species in this area are due to the nutrient-rich soil and various microclimates present in untouched pine forest, mire, river valley and sandy riverbank habitats (Fig. 3). CWR of priority food and forage crops found in these two regions include species of *Elymus*, *Festuca*, *Ribes*, *Rubus*, *Trifolium* and *Vicia*.



Figure 3 Oulanka River (Photo: Aarne Laasonen)

Site 3: Hanko peninsula

The third hotspot site is in the Hanko peninsula, in the southernmost point of mainland Finland. The total number of priority CWR found here is 51 taxa, 14 of them not found in the other five sites. The Hanko peninsula is surrounded by a large archipelago. The landscape is characterized by cliffs, fine sandy beaches and meadows (Fig. 4). Most vegetation occurs in dry pine forests on sandy soils, but some lush vegetation can also be found in areas with richer organic soils. The Hanko peninsula is known to have high species diversity with many endangered plant species. This is partially due to Hanko being an old and active harbour city, but also because of its temperate climate and the geographic location. There are several conservation areas in the Hanko peninsula and in the surrounding archipelago. CWR found in this area that are

important in terms of their relationship to food/forage crops include species of *Lathyrus*, *Vicia*, *Allium*, *Festuca*, *Trifolium*, *Atriplex*, *Salsola*, *Ribes*, *Rubus* and *Urtica*. Other valuable CWR in this area include *Elymus farctus* subsp. *boreoatlanticus*, *Malus sylvestris*, *Fragaria vesca* and *Ammophila arenaria*.



Figure 4 Coastal vegetation on Hanko peninsula in southern Finland (Photo: Jouko Lehmuskallio, Luontoportti)



Figure 5 Mountain vegetation in Lapland
(Photo: Jouko Lehmuskallio, Luontoportti)



Figure 6 Mountain stream in Lapland
(Photo: Jouko Lehmuskallio, Luontoportti)

Site 4: Saana and Malla fell area

The fourth site contains 27 priority CWR taxa, 13 of which are not found in the other hotspots. It is situated on the Saana and Malla fells on the northwest corner of the Finnish Lapland. Saana and Malla fells are located nearly 300 km above the Arctic Circle and only 50 km from the Arctic Ocean in the area where Finnish, Norwegian and Swedish parts of Lapland meet. This area is unique in many ways. The mountains are the highest in Finland and the base rock is more alkaline and younger than elsewhere in Finland. There are unusually lush growth conditions for more demanding and threatened species not found elsewhere in Finland, due to the combination of the rock type, water from melting snow throughout the summer, and the Arctic Sea influence (Figs. 5 and 6). The flora in the region is a mixture of northern boreal, alpine and arctic species, Saana and Malla Fells having the highest vascular plant diversity in the region (Järvinen and Lahti, 2004). The 10x10 km grid covers both the protected area and the herb-rich forest reserve in the Saana fell, and a part of the Malla strict nature reserve. Many threats to the species exist in the region, varying from grazing pressure, construction and recreational activities to climate change. Some examples of the priority CWR are species of *Fragaria*, *Lathyrus*, *Vicia*, *Festuca*, *Trifolium*, *Ribes* and *Rubus*.

“To prevent the decline of
populations going unnoticed,
CWR taxa need to be included
in protected area management
and monitoring plans”

Conclusion

Finland's priority CWR taxa should primarily be conserved *in situ* in their natural habitats. This allows for continued evolution and adaptation to climate change. As a backup, the priority taxa should also be collected for *ex situ* conservation. CWR utilization in breeding programmes could be facilitated through *ex situ* conservation by providing data and access to the genetic resources. As a large proportion (76%) of the Finnish flora consists of CWR in a broad sense, many of them naturally exist within protected areas. These species are, however, conserved passively. To prevent the decline of populations going unnoticed, CWR taxa need to be included in protected area management and monitoring plans. Quite a large propor-

tion (over 60%) of the Finnish priority CWR taxa would be conserved if genetic reserves were to be established in the five CWR hotspot areas. It would be beneficial to additionally look into the possibility of conserving some of the CWR populations growing outside the conservation areas, since many CWR taxa are found growing in disturbed habitats, such as roadsides, field margins, fields or wastelands. Many of these weedy species can contain important genetic resources. As Maxted and Kell (2009) state, these habitats often contain large, thriving CWR populations which can potentially provide dispersal and gene flow routes for other CWR populations, some of which may occur in protected areas. The detailed *in situ* and *ex situ* gap analyses, species lists, *ex situ* collecting recommendations and hotspot site information can be found in the National Crop Wild Relative Strategy Report for Finland (Fitzgerald, 2013) along with the CWR checklist and prioritized national CWR inventory lists.

References

- Fitzgerald, H. (2013) *The National Crop Wild Relative Strategy Report for Finland*. MTT Report 121. Jokioinen, Finland. <http://jukuri.mtt.fi/bitstream/handle/10024/481549/mttraportti121.pdf>
- Järvinen, A. and Lahti, S. (eds.) (2004) *Suurtuntureiden luonto*. Kilpisjärven biologisen aseman 40-vuotisiuhlakirja. Palmenia-kustannus, Finland.
- Lampinen, R., Lahti, T. and Heikkinen, M. (2012) *Kasviatlas 2011*. Finnish Museum of Natural History, University of Helsinki. www.luomus.fi/kasviatlas
- Maanmittauslaitos (2012) *Avoimien aineistojen tiedostopalvelu*. www.maanmittauslaitos.fi/aineistot-palvelut/latauspalvelut/avoimien-aineistojen-tiedostopalvelu
- Maxted, N. and Kell, S. (2009) *Establishment of a Network for the In Situ Conservation of Crop Wild Relatives: Status and Needs*. Commission on Genetic Resources for Food and Agriculture. Food and Agriculture Organization of the United Nations, Rome, Italy. www.fao.org/docrep/013/i1500e/i1500e18a.pdf
- SYKE (2012) *OIVA – Ympäristö – ja paikkatietopalvelu asiointitijoiille*. <http://www.p2.ymparisto.fi/scripts/oiva.asp>

In situ conservation of CWR in Spain: present and future

M.L. Rubio Teso¹, C. Ronquillo Ferrero¹, A. Nebreda Trejo¹, M. Parra Quijano², E. Torres Lamas³ and J.M. Iriondo Alegría¹

¹Departamento de Biología y Geología, Física y Química Inorgánica. Universidad Rey Juan Carlos, C/Tulipán, s/n. E-28933 Móstoles (Madrid), Spain. Email: marialuisa.rubio@urjc.es

²International Plant Treaty for Food and Agriculture Division. Food and Agricultural Organization of the United Nations, Via delle Terme di Caracalla, 00153 Rome, Italy.

³Departamento de Biología Vegetal. Escuela Universitaria de Ingeniería Técnica Agrícola, Avda de la Complutense, s/n. E-28040 Madrid, Spain.

One of the tasks carried out under the frame of the PGR Secure project was the development of national strategies for the preservation of CWR across Europe. These national strategies have produced lists of prioritized CWR, inventories and also studied the *in situ* and *ex situ* conservation status of the CWR selected, as well as generated proposals for better conservation and better access to them (Fitzgerald, 2013; Rubio Teso *et al.*, 2013; Panella *et al.*, 2014).

The baseline national CWR checklist for Spain contains more than 6500 species (Kell *et al.*, 2008). In the context of PGR Secure project the prioritization process of Spanish CWR resulted in a final list of 580 species that compose the National Inventory of Crop Wild Relatives for Spain (Rubio Teso *et al.*, 2013). However, lists of species are not enough to propose actions that effectively preserve these resources. Data on the *in situ* and *ex situ* conservation of CWR are necessary to properly understand the status of these species in the country and develop efficient measures to enable optimal conservation and use. The *ex situ* conservation of plant species plays an important role in conservation plans because it allows the preservation of a large number of seed accessions in reduced space and can prevent the loss of genetic diversity for many species (Bacchetta *et al.*, 2008). On the other hand, and complementary to *ex situ* preservation, the *in situ* conservation of CWR is outlined as the best way to preserve plant genetic resources as it enables dynamic maintenance of the genetic diversity of the species (Heywood and Dulloo, 2005) by allowing populations to evolve in their natural community (Prance, 1997).

Methodology and results

The prioritized list of 580 CWR species was subdivided into four categories according to the use of the reference crops: food, forage and fodder, ornamental and industrial and other uses. Distribution data for 553 species were available and downloaded from the Global Biodiversity Information Facility

data portal (data.gbif.org). After a taxonomic harmonization following the Spanish reference flora (*Flora Iberica*, Castroviejo *et al.*, 1986–2012) and the Anthos project (www.anthos.es), all records were subjected to meticulous quality screening. Only those which accomplished the minimum standards established—1) geographic coordinates that, expressed in decimal degrees, hold at least two decimal digits, and 2) full information on territorial and local administrative units where the population is located—were used for the analysis. After the elimination of duplicates and, when possible, elimination of data records of cultivated forms, geographic and ecogeographic gap analyses were performed.

All data, together with layers of political boundaries of Spain, polygons of the sites of community interest (SCI) in the Natura 2000 network of Spain and an ecogeographical land characterization (ELC) map for the Iberian Peninsula and Balearic Islands (Parra Quijano *et al.*, 2012) were introduced into a Geographic Information System (GIS) using ArcGIS version 10.0 (ESRI, 2010). The ELC map for Spain used for this work was built combining climatic, geophysical and edaphic variables that represent the different environments in Spain. On the basis that different environmental conditions may result in diverse selection pressures, we can assume that unique adaptive genetic diversity (specific adaptations to specific environments) may be found for each species in each ecogeographical unit in which a species can be found. Thus, ELC maps can be used as a proxy to estimate genetic diversity (Maxted *et al.*, 2012).

A gap analysis between the species occurrence data layer and the Natura 2000 layer was carried out in order to assess how many of the known occurrences for each species were under passive protection. Around half of the records— $42 \pm 24\%$ (Mean \pm Standard Deviation)—were located inside protected areas belonging to the Natura 2000 network. When results were taken separately by use categories, the higher value was achieved in the forage and fodder group ($47 \pm 23\%$ ($\mu \pm$ SD)),



Patellifolia procumbens population in Rambia de Castro (Los Realejos), Tenerife. Canary Islands (Spain) (Photo: Yurena Arjona Fariña)

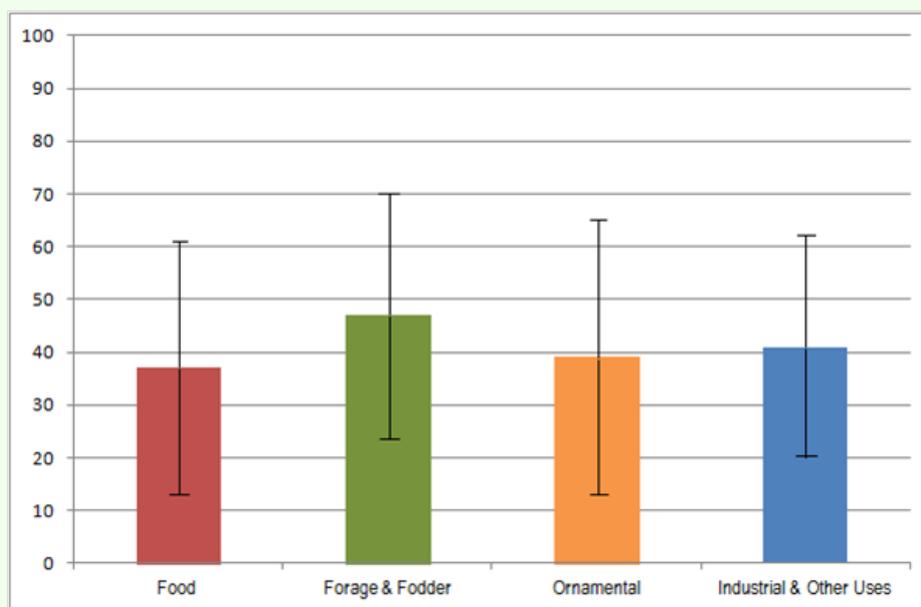


Figure 1 Percentage of prioritized CWR populations occurring in protected areas (Sites of Community Interest in the Natura 2000 network of Spain) per use category (mean values \pm standard deviations)

followed by the industrial and other uses category ($41 \pm 21\%$ ($\mu \pm SD$)), the ornamental category ($39 \pm 26\%$ ($\mu \pm SD$)) and the food category ($37 \pm 24\%$ ($\mu \pm SD$)) (Fig. 1). Expressed in other terms, 471 species ($\approx 81\%$) have at least one population in a protected area, but if it is considered that a minimum number of five populations is needed to ensure a good protection of the species (Maxted *et al.*, 2008), 372 species ($\approx 64\%$) fulfil this condition. It is worth mentioning that, according to currently available data, 38 species under analysis had not even a single population within the limits of the Natura 2000 network, but conversely, 18 species held all their known populations under passive protection.

A second gap analysis was carried out adding to the former data layers a new data layer with the ecogeographic unit data value corresponding to each occurrence record. Taking into account that in the case of Spain the number of different ecogeographic units in which a species is distributed can be up to 27, $66 \pm 27\%$ ($\mu \pm SD$) of the ecogeographic units where the prioritized CWR species of the Iberian Peninsula and Balearic Islands are found are represented in the populations that occur in protected areas in the Natura 2000 network. In terms of species, 215 ($\approx 37\%$) have more than 75% of their ecogeographic units under passive protection in the Natura 2000 network and 105 of these ($\approx 18\%$) more than 90%.

Discussion and implications for conservation

If aimed at achieving the conservation of at least two thirds of the prioritized CWR species (Maxted *et al.*, 2007), the Natura 2000 network in Spain is close to fulfilling this goal (64% of the species have at least five populations in the Natura 2000 network). However, although the overall representation of ecogeographic units in which the species are represented (66%) is high, only 37% of the species have more than 75% of their potential genetic diversity of adaptive value included in the Natura 2000 network. The conservation of genetic diversity of adaptive value is a key factor when facing crop adaptation to climate change. It is thus necessary to preserve the maximum

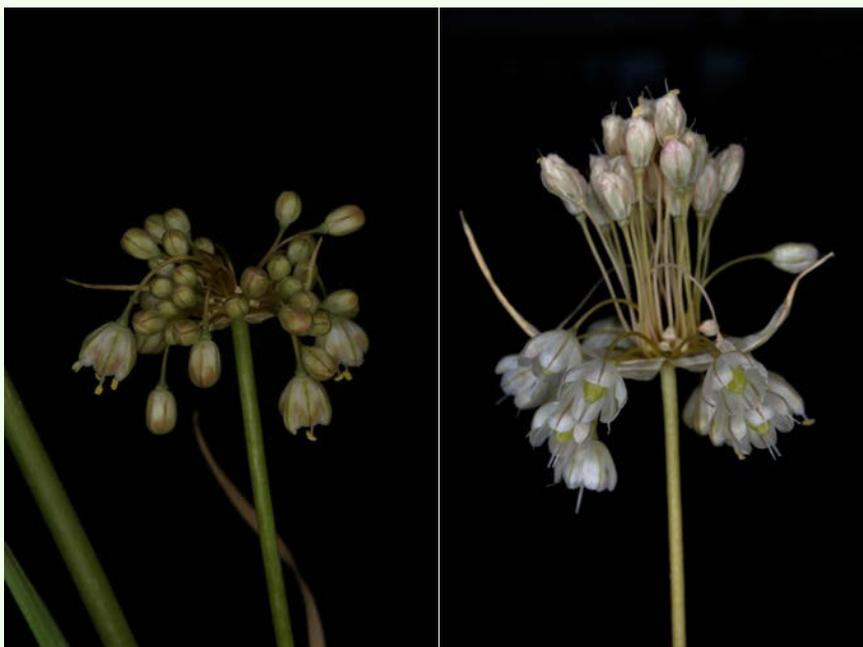
adaptive diversity in order to guarantee a wide pool of genes available for plant breeding.

The high standard deviations obtained in the analyses show the heterogeneity of the data used and also the diverse distribution ranges of species under study. It is difficult to obtain good quality data for all species, especially for those which are not very well studied—for example because, paradoxically, they are very common. Examples include *Allium stearni* Pastor & Valdés which is distributed across over 21 out of 50 prov-

“The conservation of genetic diversity of adaptive value is a key factor when facing crop adaptation to climate change”



Astragalus cavaniillesii in Granada province, southern Spain (Photo: José A. Algarra)



Inflorescences of *Allium stearni*. Pictures taken in the marsh of Pego-Oliva, between the provinces of Alicante and Valencia, in eastern Spain (Photo: Emilio Laguna)

inces in Spain and only holds one data record and *Patellifolia procumbens* (C. Sm. ex Hornem.) A. J. Scott, Ford-Lloyd & J. T. Williams which is distributed in all the Canary Islands but with only two high-quality georeferenced occurrences. The other reason that explains these elevated standard deviations is that many of these species have very narrow distributions and the few existing populations are not found within the Natura 2000 network. For example, *Astragalus cavanillesi* Podlech, a CWR which is Critically Endangered in Spain and in Taxon Group 3 (see Taxon Group concept in Maxted *et al.*, 2006) of the fodder crop *Astragalus glycyphyllos* L.

Results from this study outline the importance of adequately conserving the genetic diversity as well as the species diversity of prioritized CWR. ELC maps are a powerful tool to assess the potential genetic diversity of adaptive value. Following this approach, target conservation units are composed of populations representing each ecogeographic unit where a prioritized CWR occurs. The use of the new target unit of conservation (CWR species-ecogeographic unit combination), together with the utilization of complementarity analysis—conservation of the maximum number of conservation units in the minimum number of sites—can be particularly useful for the identification of potential genetic reserves. This approach provides an efficient method to identify areas with special value for CWR conservation both in terms of the number of species and useful adaptive potential.

References

- Bachetta, G., Bueno Sánchez, A., Fenu, G., Jiménez-Alfaro, B., Matana, E., Piotto, B. and Virevare, M. (eds.) (2008) *Conservación Ex Situ de Plantas Silvestres*. Principado de Asturias / La Caixa.
- Castroviejo S., (coord. gen.) (1986–2012) *Flora Iberica* 1–8, 10–15, 17–18, 21. Real Jardín Botánico, CSIC, Madrid.
- ESRI (2011) *ArcGIS Desktop Release 10*. Environmental Systems Research Institute, Redlands CA.
- Fitzgerald, H. (2013) *The National Crop Wild Relative Strategy Report for Finland*. MTT Report 121. Jokioinen, Finland. <http://jukuri.mtt.fi/bitstream/handle/10024/481549/mttraportti121.pdf>
- Heywood, V.H. and Dulloo, M.E. (2005) *In Situ Conservation of Wild Plant Species: A Critical Global Review of Best Practices*. IPGRI Technical Bulletin 11. IPGRI, Rome, Italy.
- Kell, S.P., Knüpffer, H., Jury, S.L., Ford-Lloyd, B.V. and Maxted, N. (2008) Crops and wild relatives of the Euro-Mediterranean region: making and using a conservation catalogue. In: Maxted, N., Ford-Lloyd, B.V., Kell, S.P., Iriondo, J., Dulloo, E. and Turok, J. (eds.) *Crop Wild Relative Conservation and Use*. CABI Publishing, Wallingford. Pp. 69–109.
- Maxted, N., Dulloo, E., Ford-Lloyd, B.V., Iriondo, J.M. and Jarvis, A. (2008) Gap analysis: a tool for complementary genetic conservation assessment. *Diversity and Distributions* 14: 1018–1030.
- Maxted, N., Ford-Lloyd, B.V., Jury, S., Kell, S. and Scholten, M. (2006) Towards a definition of a crop wild relative. *Biodiversity and Conservation* 15: 2673–2685.
- Maxted, N., Kell, S., Ford-Lloyd, B., Dulloo, E. and Toledo, Á. (2012) Toward the systematic conservation of global crop wild relative diversity. *Crop Science* 52: 774–785.
- Maxted, N., Scholten, M., Codd, R. and Ford-Lloyd, B. (2007) Creation and use of a national inventory of crop wild relatives. *Biological Conservation* 140: 142–159.
- Panella, L., Landucci, S., Torricelli, R., Gigante, D., Donnini, D., Venanzoni, R. and Negri, V. (2014) *The National Crop Wild Relative Strategy for Italy: First Steps To Be Taken*. PGR Secure. www.pgrsecure.bham.ac.uk/sites/default/files/documents/public/National_CWR_Conservation_Strategy_Italy.pdf
- Parra Quijano, M., Iriondo, J.M. and Torres, E. (2012) Ecogeographical land characterization maps as a tool for assessing plant adaptation and their implications in agrobiodiversity studies. *Genetic Resources and Crop Evolution* 59(2): 205–217.
- Prance, G.T. (1997) The conservation of botanical diversity. In: Maxted, N., Ford-Lloyd, B.V. and Hawkes, J.G. (eds.) *Plant Genetic Conservation: The In Situ Approach*. Chapman & Hall, London (UK). Pp. 1–11.
- Rubio Teso, M.L., Iriondo, J.M., Parra Quijano, M. and Torres, E. (2013) *National Strategy for the Conservation of Crop Wild Relatives of Spain*. PGR Secure. [online] Available at: www.pgrsecure.bham.ac.uk/sites/default/files/documents/public/National_CWR_Conservation_Strategy_Spain.pdf

Italian crop wild relatives and wild harvested plants conservation strategy

L. Panella¹, F. Landucci^{1,2}, D. Donnini¹, D. Gigante^{1,3}, R. Venanzoni^{1,3}, L. Raggi¹, R. Torricelli¹ and V. Negri¹

¹Department of Agriculture, Nutritional and Environmental Sciences, University of Perugia, Borgo XX Giugno 74, 06121 Perugia, Italy.

²Present address: Department of Botany and Zoology, Masaryk University, Kotlářská 2, 61137 Brno, Czech Republic.

³Present address: Department of Chemistry, Biology and Biotechnology, University of Perugia, via Elce di Sotto 8, 06123 Perugia, Italy.

In the frame of the PGR Secure project we operated in order to build an Italian CWR inventory and to improve the knowledge of the taxonomic, distribution and ecological features of these taxa. We considered a CWR following both the Taxon Group and the Gene Pool concepts (Harlan and de Wet, 1971; Maxted *et al.*, 2006) and a wild harvested species (WHP) following Magos Brehm *et al.* (2008) (i.e. any non-cultivated species which is collected from the wild and used by local people).

Italian CWR checklist and priority lists

The initial step to develop the conservation strategy was to create a checklist of CWR and WHP and then to select priority species for conservation planning. This was achieved through three steps:

1. We inventoried all the CWR and WHP species of Italy, recording for each of them: synonymies, uses, presence in the main areas of Italy (i.e. Peninsula, Sicily and Sardinia), presence in the European and National lists of attention, precisely European Red List (Bilz *et al.*, 2011), National and Regional Red Lists (Conti *et al.*, 1992, 1997;

Rossi *et al.*, 2013) and Bern Convention (Council of Europe, 1979).

2. We created a priority list of Italian CWR/WHP using the following prioritization criteria: relation with food crop (Annex I ITPGRFA – FAO, 2001; ISTAT, 2012), autochthony and inclusion in European and National Lists of attention (Council of Europe, 1979; Conti *et al.*, 1992, 1997; Bilz *et al.*, 2011; Rossi *et al.*, 2013).
3. We carried out a further prioritization process at Administrative Regions level, in two case studies: Sicily and Sardinia, the two largest Italian islands. For these Regions, the inclusion of the species in the Regional Red Lists (Conti *et al.*, 1997) was considered as an additional criterion for prioritization.

The CWR/WHP checklist of Italy contains 7128 species (Landucci *et al.* 2014; see also <http://vnr.unipg.it/PGRSecure/>). The Italian priority list includes 797 species of which 123 are top priority, because they are related to food crops (Annex I ITPGRFA – FAO, 2001; ISTAT, 2012), autochthons, and included in European and National Lists of attention (Council of Europe, 1979; Conti *et al.*, 1992, 1997; Bilz *et al.*, 2011; Rossi *et al.*, 2013). *Allium* L. and *Brassica* L., in particular, have the



Figure 1 A wild *Brassica incana* population in Tuscany (Photo: Flavia Landucci)



Figure 2 Prof. Venanzoni assessing a population of *B. incana* in central Italy (Photo: Roberto Venanzoni)

highest number of endemic taxa and deserve immediate protection measures.

The Sicilian and Sardinian priority lists include 74 and 43 species, respectively, deserving the highest attention in planning a PGR conservation strategy.

This work is only the initial step to develop a CWR/WHP conservation strategy. In future it will be necessary to: a) provide precise information on actual occurrence, location and census of

CWR/WHP populations starting from top priority species; b) identify CWR/WHP populations that are not included in any protected areas and are not safely preserved *ex situ*; c) draw appropriate management plans for extant CWR/WHP populations that are present *in situ* in protected areas; and d) collect seed samples for safe *ex situ* conservation.

Gap analysis case studies

Brassica CWR are native of the Mediterranean Basin and closely related to many important crops. In Italy, according to the Italian CWR checklist (Landucci *et al.*, 2014; see also <http://vnr.unipg.it/PGRSecure/>), there are 19 species and 52 subspecific taxa of *Brassica*. Information on their distribution and their conservation status is presently lacking. To gather initial information we carried out *in situ* and *ex situ* gap analyses for two case study CWR: *Brassica incana* Ten. (Fig. 1) and *B. montana* Pourr., which are wild species closely related to *B. oleracea* L.

Distribution data were collected from the literature (Pignatti, 1982), gene bank records (EURISCO – <http://eurisco.ecp-gr.org/> and ECPGR *Brassica* Database – <http://documents.plant.wur.nl/cgn/pgr/brasedb/>) and personal communications. Some locations of central Italy were visited and actual population occurrences assessed (Fig. 2).

The gap analysis process was carried out considering:

1. Populations included in protected areas, i.e.:
 - Natura 2000 Sites of Community Importance (SCI) (European Commission, 1995–2007)
 - Natura 2000 Special Protection Areas (SPA) (European Commission, 1995–2007)
 - Areas mentioned in the ‘Elenco Ufficiale delle Aree Naturali Protette’ (EUAP) (Ministero dell’Ambiente e della Tutela del Territorio e del Mare, 2011)
2. Populations collected and conserved in gene banks.

We recorded 48 and 41 populations of *B. incana* and *B. montana*, respectively in central Italy. During field visits, some of them could not be found, probably because they were not well

located in the past or are now extinct. Most of the populations of both species appear to be included in some type of protected areas and benefit from a (at least passive) form of *in situ* protection. On the other hand, populations secured in gene banks are 40 for *B. incana* and only 16 for *B. montana*.

This study is just a first step in increasing knowledge about CWR of crops of great importance like *Brassica*. Very little is known about them (as well as about CWR of other important crops) in Italy and further studies are needed to plan and implement their effective and efficient conservation in the future.

References

- Bilz, M., Kell, S.P., Maxted, N. and Lansdown, R.V. (2011) *European Red List of Vascular Plants*. European Union, Luxembourg. http://ec.europa.eu/environment/nature/conservation/species/redlist/downloads/European_vascular_plants.pdf
- Conti, F., Manzi, A. and Pedrotti, F. (1992) *Libro Rosso Delle Piante d’Italia*. WWF Italia, Società Botanica Italiana. Poligrafica Editrice, Roma.
- Conti, F., Manzi, A. and Pedrotti, F. (1997) *Liste Rosse Regionali Delle Piante d’Italia*. WWF Italia, Società Botanica Italiana. TIPAR Poligrafica Editrice, Camerino.
- Council of Europe (1979) *Bern Convention – The Convention on the Conservation of European Wildlife and Natural Habitats*. CoE, Strasbourg.
- European Commission (1995–2007) *Council Directive 92/43/EEC of 21 May 1992 on the Conservation of Natural Habitats and of Wild Fauna and Flora*. <http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31992L0043:EN:HTML>
- FAO (2001) *The International Treaty on Plant Genetic Resources for Food and Agriculture*. www.planittreaty.org/
- Harlan, P. and de Wet, J.M.J. (1971) Towards a rational classification of cultivated plants. *Taxon* 20: 509–517.
- ISTAT (2012) Istituto Nazionale di Statistica. www.istat.it/it/
- Landucci, F., Panella, L., Lucarini, D., Gigante, D., Domizia, D., Kell, S., Maxted, S., Venanzoni, R. and Negri, V. (2014) A prioritized inventory of crop wild relatives and wild harvested plants of Italy. *Crop Science*. DOI: 10.2135/cropsci2013.05.0355.
- Magos Brehm, J., Maxted, N., Ford-Lloyd, B.V. and Martins Loução, M.A. (2008) National inventories of crop wild relatives and wild harvested plants: case study for Portugal. *Genetic Resources and Crop Evolution* 55: 779–796.
- Maxted, N., Ford-Lloyd, B.V., Jury, S.L., Kell, S.P. and Scholten, M.A. (2006) Towards a definition of a crop wild relative. *Biodiversity Conservation* 15(8): 2673–2685.
- Ministero dell’Ambiente e della Tutela del Territorio e del Mare (2011) *Elenco ufficiale aree protette (EUAP)*. www.pcn.minambiente.it/viewer/index.php?services=EUAP&box=78421.38684277324.4342343.1546863085.566578.6131572267.4937656.845313691
- Pignatti, S. (1982) *Flora d’Italia*. Edagricole. Bologna.
- Rossi, G., Montagnani, C., Gargano, D., Peruzzi, L., Abeli, T., Ravera, S., Cogoni, A., Fenu, G., Magrini, S., Gennai, M., Foggi, B., Wagensommer, R.P., Venturella, G., Blasi, C., Raimondo, F.M. and Orsenigo, S. (eds.) (2013) *Lista Rossa della Flora Italiana. 1. Policy Species e altre specie minacciate*. Comitato Italiano IUCN e Ministero dell’Ambiente e della Tutela del Territorio e del Mare.

Plant Genetic Resources Diversity Gateway

for the conservation and use of crop wild relative and landrace traits

S. Dias

Bioversity International, Via dei Tre Denari 472/a, Rome, Italy. Email: s.dias@cgiar.org

One comprehensive information gateway for plant genetic resources for food and agriculture

The Plant Genetic Resources Diversity Gateway (the PGR Diversity Gateway) (Fig. 1) was developed during the PGR Secure project and aims to promote and facilitate the use of crop wild relatives and landraces in breeding and crop improvement by providing trait and quantitative trait locus (QTL) information of potential value to breeders and other users of germplasm. The system went online in July 2014 and accommodates different data types in one place, including nomenclatural, biological, ecogeographical, conservation and trait data, as well as checklists, conservation strategies and a mapping service (Fig. 2). It paves the way for maintaining the wealth of data and information gathered during research activities, which otherwise often become lost within a few years of the end of the research.

The objectives of the PGR Diversity Gateway are to:

- Help institutions, national and international agencies, NGOs, training and research groups throughout the world to share data on crop wild relatives and landraces and coordinate efforts for better policy, management and facilitated use of information and natural resources.
- Achieve better management, conservation and use of all crop wild relative and landrace genetic resources for better and more sustainable agricultural production of food in all countries, in accordance with the [World Food Summit Plan of Action](#), the [International Treaty on Plant Genetic Resources for Food and Agriculture](#) and the [UN Convention on Biological Diversity](#).
- Offer plant breeders more and better information on traits, crop wild relatives and landraces.

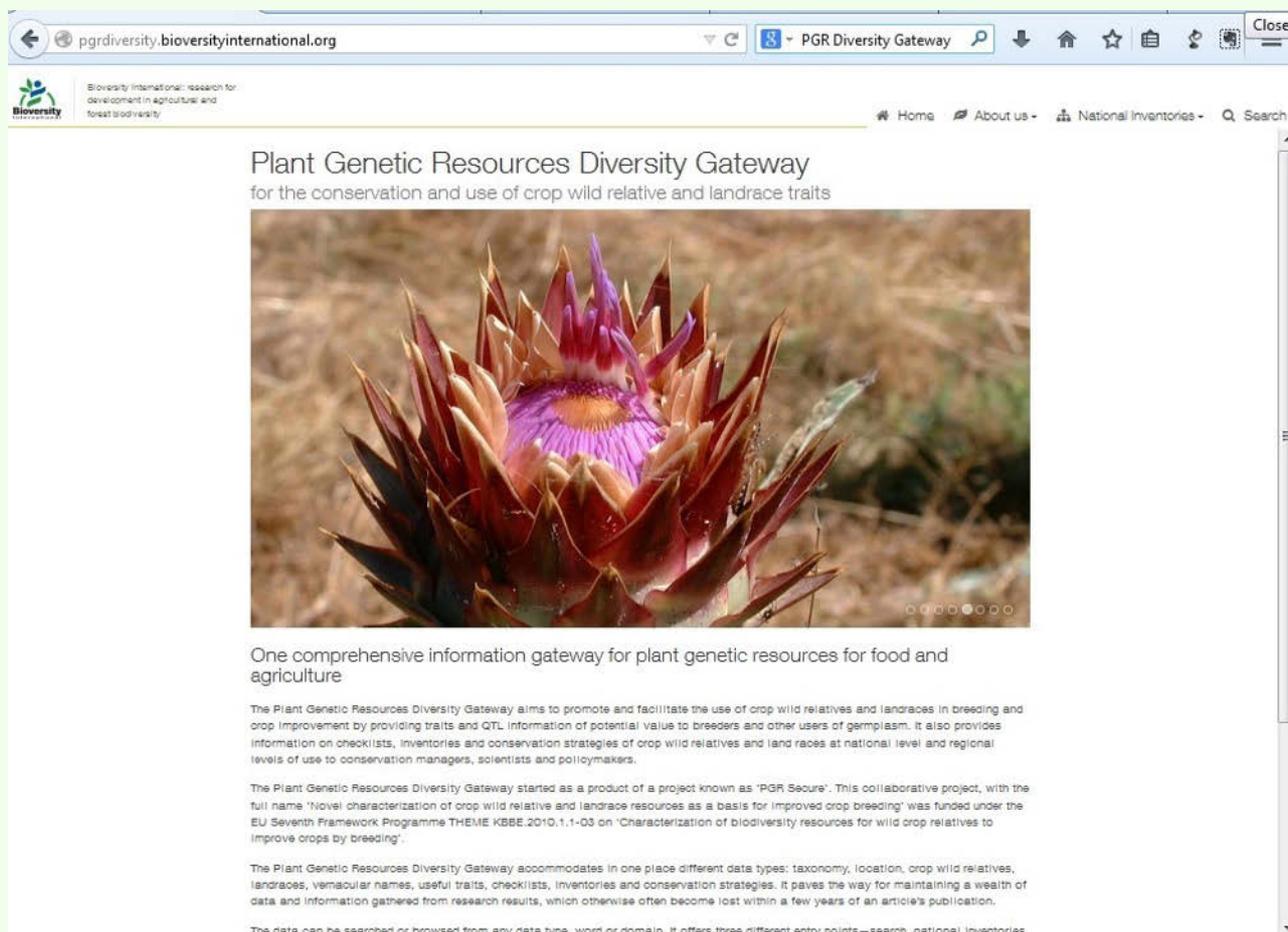


Figure 1 PGR Diversity Gateway home page view

The PGR Diversity Gateway offers:

- A ready-to-use platform with a portal and visualization map service.
- A means to maintain, access and share data.
- An advanced communication and information tool to facilitate country reporting and policy decision-making on PGRFA.
- An infrastructure for storing and linking data on crop wild relatives, landraces, traits, QTL, evaluation data, environmental data, inventories, checklists and conservation strategies (Fig. 3).
- A central point for country, regional and global contacts on crop wild relative and landrace information.

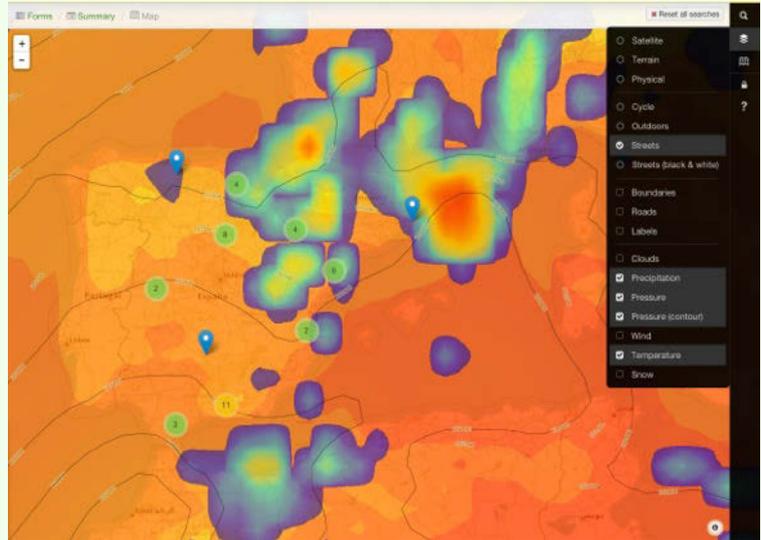


Figure 2 Map view – clustered distribution overlaid on a map with pressure contour, pressure and precipitation layers

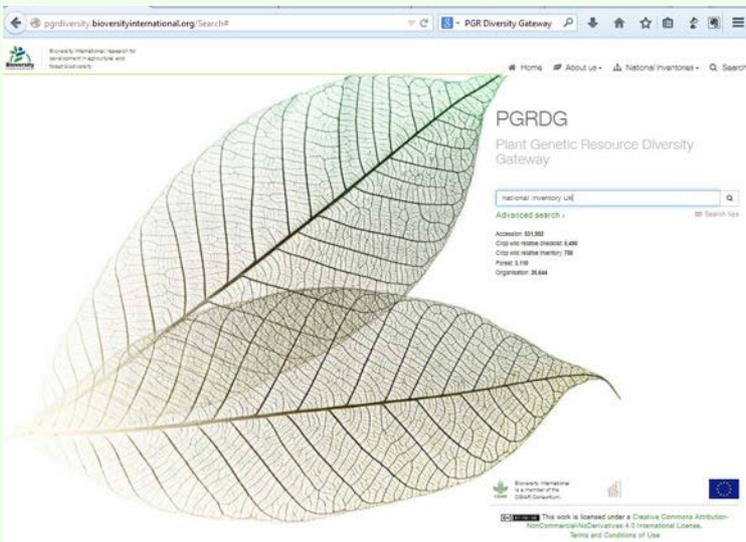


Figure 3 View of the search page with the generic and advanced search features

The data can be searched or browsed from any data type, word or domain. The PGR Diversity Gateway offers three different entry points, allowing users to retrieve information without having to choose a domain beforehand: i) generic and advanced search options, ii) national inventories, and iii) conservation strategies (Fig. 4).

Visit the Gateway at: <http://pgrdiversity.bioversityinternational.org>



Figure 4 System layers – from the actual data to the user interface

Hordeum vulgare subsp. *spontaneum* in Turkey: characterization in a laboratory environment

F. Gürel¹, C. Uçarlı¹, E. Yörük¹ and A. Karagöz²

¹ Istanbul University, Department of Molecular Biology and Genetics, Vezneciler 34134, Istanbul, Turkey. Email: filiz@istanbul.edu.tr

² Aksaray University, Vocational School for Technical Sciences, 68100, Aksaray, Turkey

Turkey has a large diversity of plant species due to its great variety in geomorphology, topography and climate (Karagöz *et al.*, 2010). *Hordeum vulgare* L. subsp. *spontaneum* (C. Koch.) Thell. which is the progenitor of cultivated barley (*H. vulgare*) (Harlan and Zohary, 1966; von Bothmer and Jacobsen, 1985) is an annual, two-rowed diploid species with $2n=2x=14$ chromosomes and a diploid genome size of 9.03pg (Kalendar *et al.*, 2000). The primary distribution of the taxon is in southeastern Anatolia as part of the Fertile Crescent, but it is also distributed in northern and western regions of the country (Davis *et al.*, 1985).



Figure 1 One of the locations of *H. vulgare* subsp. *spontaneum* sampling near Hasankeyf, Batman (Photo: Lokman Baş)

H. vulgare subsp. *spontaneum* is an important source of genetic variation for barley breeding since there is no barrier for crossing with *H. vulgare* (Nevo, 1991; Ceccarelli *et al.*, 2004). High genetic variation based on RAPD markers has already been demonstrated even in small numbers of *H. vulgare* subsp. *spontaneum* populations located in the Near East (Baum *et al.*, 1997). Polymorphism in isolated populations of *H. vulgare* subsp. *spontaneum* in Turkey was determined as nearly 50% according to Nei and Li (1979) by using arbitrary primers (Gürel ve Gözükırmızı, 1998; Albayrak and Gözükırmızı, 1999). Notably, high genetic diversity has been revealed by sampling more distinct populations rather than excess individuals within a population (Özkan *et al.*, 2005). Genetic variation is linked with the existence of a wide range of environments in the Middle East with differing altitudes, rainfalls, temperatures, soil types and photoperiods (Forster *et al.*, 1997). A large-scale genetic diversity analysis of collected germplasm in Turkey is still needed. In addition, phenotypic variability of *H. vulgare* subsp. *spontaneum* to environmental changes is crucial to assess plant responses at whole-plant level. Therefore, any data on phenotypic and physiological responses will greatly contribute to our understanding on wide adaptability of this taxon.

Barley breeding has been carried out in rainfed marginal environments in Turkey and yield is usually affected by climatic changes. Integration of agronomically valuable characters including abiotic stresses to cultivated barley is a globally important issue. Abiotic stress tolerance and disease resistance of wild barley populations of Anatolian origin have not been well characterized. We conducted a research project (IU BAP. 4712) in collaboration with the Aegean Agricultural Research Institute (AARI, Izmir) during 2010–2012 to assess physiological and molecular responses of both elite varieties and *H. vulgare* subsp. *spontaneum* to salinity and water stress. Germination of wild barley is an important step for growth and propagation in a laboratory environment. Seed dormancy particularly limits germination and seedling growth which is necessary for subsequent applications of experimental stresses to simultaneously grow individual plants. During the project, we examined germination percentage, growth characteristics and physiological stress responses of 29 *H. vulgare* subsp. *spontaneum* accessions. Seeds of 12 accessions originating from Çanakkale, Adıyaman, Diyarbakır, Şanlıurfa, Siirt, Gaziantep, Izmir and Mardin provinces were provided by the AARI gene bank and 17 accessions were collected by our group from Hasankeyf (Batman), Bismil and Diyarbakır. These accessions have been sampled from the populations located in different altitudes and distances to Dicle River (Fig. 1). Seeds were

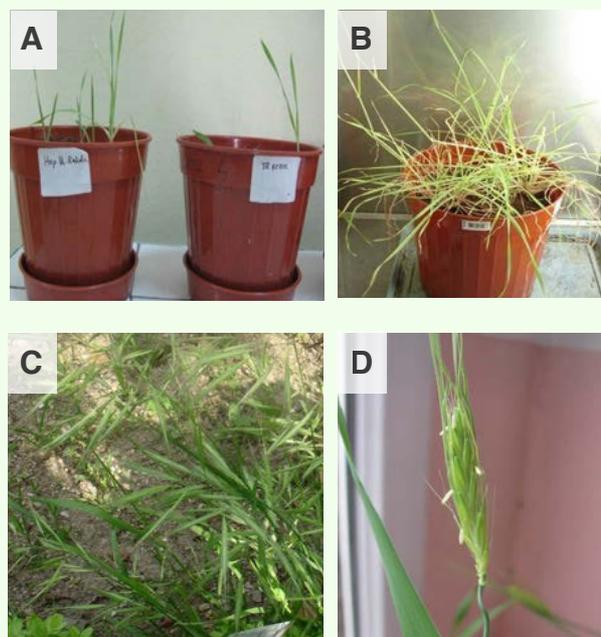


Figure 2 (A) Seedling development in gene bank accessions (TR4982/Çanakkale and TR47002/Izmir) after ten weeks at room temperature; (B) Seedling development in TR31623/Mardin in a plant growth chamber (25°C, relative humidity 40%, 16 h light/ 8 h dark photoperiod and light intensity of 600 Moles/m²/s); (C) Flowering plants growing in the field; (D) Flowering in laboratory conditions.

directly sown in pots or pre-germinated in wet filter papers. Another set of seeds were sown in the field of IU Department of Botany over three years. The germination percentage of entire populations ranged between 16–85%. Seedling growth was altered in accessions by either induction of tillering or inducing long shoots (Figs. 2A, B). Flowering occurred in natural periods (about five months) after sowing in the field and pots in the laboratory (Figs. 2C, D). As fast screening methods, relative water content (RWC) and water loss rate (WLR) determinations were used to measure the water content of genotypes under ten days of water stress. RWCs in the leaves of five *H. vulgare* subsp. *spontaneum* lines (TR-40812, TR-31623, TR-47002, TR-49085, MBG-HB1) varied between 67–79% and 47–68% in control and stressed plants, respectively. WLR of the same *H. vulgare* subsp. *spontaneum* lines ranged between 0.1136 to 0.2034 $\text{gh}^{-1}\text{g}^{-1}\text{DW}$. Salinity stress was applied to the seedlings of 2–3 fully emerged stems (Fig. 3A) by transferring the plants from perlite to hydroponics containers including 200mM NaCl. Expression patterns of antioxidant genes have been determined by reverse transcriptase PCR (RT-PCR) in *H. vulgare* subsp. *spontaneum* line (LH1) during 0–5h after exposure to salinity (Fig. 3B).

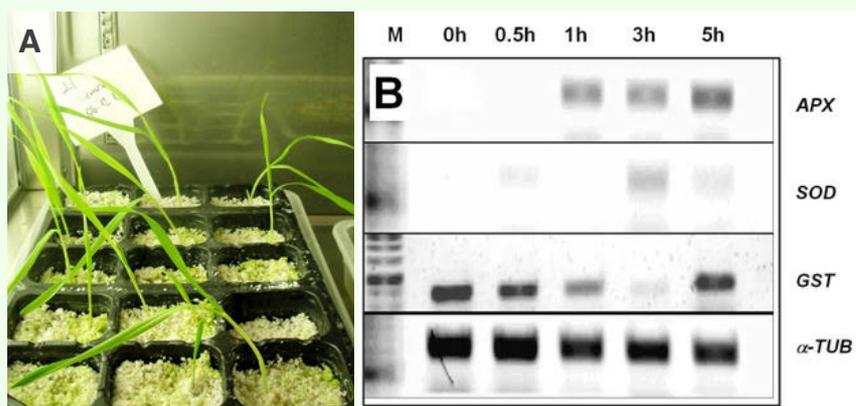


Figure 3 (A) Seedlings of the LH1 line have been used for salinity application; (B) Expression of ascorbate peroxidase (APX), superoxide dismutase (SOD), glutathione S-transferase (GST) and housekeeping gene α -tubuline (α -TUB) in the leaves of seedlings growing in hydroponics with 200 mM NaCl

“habitat fragmentation due to crop cultivation and heavy grazing are the main causes for loss of *Hordeum* biodiversity”

H. vulgare subsp. *spontaneum* accessions exist in several gene banks throughout the world. However, *ex situ* and *in situ* conservation of this species, particularly in southeastern Turkey should be re-considered and extended by national research programmes. Several sites have been assigned for *in situ* conservation of *H. vulgare* subsp. *spontaneum* in Ceylanpınar State Farm of Şanlıurfa province. Potential new hotspots of *Hordeum* diversity should be determined. Our observation is that habitat fragmentation due to crop cultivation and heavy grazing are the main causes for loss of *Hordeum* biodiversity around Hasankeyf and Bismil areas. In conclusion, our studies will continue to characterize available germplasm in order to obtain more specific data.

References

- Albayrak, G. and Gözükmızı, N. (1999) RAPD Analysis of genetic variations in barley. *Turkish Journal of Agriculture and Forestry* 23: 627–630.
- Baum, B.R., Nevo, E., Johnson, D.A. and Beiles, A. (1997) Genetic diversity in wild barley (*Hordeum spontaneum* C. Koch) in the Near East: a molecular analysis using Random Amplified Polymorphic

- DNA (RAPD) markers. *Genetic Resources and Crop Evolution* 44: 147–157.
- Ceccarelli, S., Grando, S., Baum, M. and Udupa, S. (2004) Breeding for drought resistance in a changing climate. In: Rao, S.C. and Ryan, J. (eds.), *Challenges and Strategies for Dryland Agriculture*. Crop Science Society of America and American Society of Agronomy, Wisconsin, USA. Pp. 167–190.
- Davis, P.H., Mill, R.R. and Tan, K. (1985) *Flora of Turkey and East Aegean Islands*. Version 9, Edinburgh University Press, UK.
- Forster, B.P., Russell, J.R., Ellis, R.P., Handley, L.L., Robinson, D., Hackett, C.A., Nevo, E., Waugh, R., Gordon, D.C., Keith, R. and Powell, W. (1997) Locating genotypes and genes for abiotic stress tolerance in barley: a strategy using maps, markers and the wild species. *New Phytologist* 137: 141–147.
- Gürel, F. and Gözükmızı, N. (1998) RAPD polymorphism between wild lines of barley (*Hordeum vulgare* L.) originated from Turkey. *Genetics and Breeding* 29(1–4): 19–23.
- Harlan, J.R. and Zohary, D. (1966) Distribution of wild wheats and barley. *Science* 130: 1074–1080.
- Kalendar, R., Tanskanen, J., Immonen, S., Nevo, E. and Schulman, A.H. (2000) Genome evolution of wild barley (*Hordeum spontaneum*) by BARE-1 retrotransposon dynamics in response to sharp microclimatic divergence. *Proceedings of the National Academy of Sciences of the United States of America* 97(12): 6603–6607.
- Karagöz, A., Zencirci, N., Tan, A., Taşkın, T., Köksel, H., Sürek, M., Toker, C. and Özbek, K. (2010) Conservation and utilization of plant genetic resources. *Proceedings of Turkey Agricultural Engineering VIIth Technical Congress*, 11–15 January 2010. V I, 155–177.
- Nei, M. and Li, W.H. (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the United States of America* 76(10): 5269–5273.
- Nevo, E. (1991) Origin, evolution, population genetics and resources for breeding of wild barley, *Hordeum spontaneum*, in the fertile crescent. In: Shewry, P. (ed.), *Barley: Genetics, Biochemistry, Molecular Biology and Biotechnology*. CABI Publishing, Wallingford. Pp. 19–43.
- Özkan, H., Kafkas, S., Sertaç Özer, M. and Brandolini, A. (2005) Genetic relationships among South-East Turkey wild barley populations and sampling strategies of *Hordeum spontaneum*. *Theoretical and Applied Genetics* 112(1): 12–20.
- von Bothmer, R. and Jacobsen, N. (1985) Origin, taxonomy, and related species. In: Rasmusson, D. (ed.), *Barley*. ASA Monograph No. 26. Pp.19–56.

Enhancing the conservation and use of *Medicago* genetic resources by means of targeted collection using next-generation sequencing

H. Fielder, B. Ford-Lloyd and N. Maxted

School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK. Email: hvf708@bham.ac.uk

Crop wild relatives (CWR) are poorly represented in *ex situ* collections (Dias *et al.*, 2012). It is vital that these collection gaps are filled, not only through collection of samples from the different species, but also through collection of samples covering the maximum range of genetic diversity found across the range of these wild species. By systematically conserving genetically diverse samples of CWR, more material with potentially beneficial traits can be made available for use in crop improvement in a resource and time efficient manner. In order to achieve the targeted collection of CWR material it will be necessary to directly analyse the genetic diversity of current accessions to identify gaps in collections and ultimately the sites likely to harbour further complementary diversity.

In the current study, we have applied 'next-generation sequencing' for whole genome resequencing of 13 ecogeographically diverse accessions of *Medicago truncatula* to demonstrate its use in targeting collection. *M. truncatula* is a CWR of alfalfa (*Medicago sativa*), an economically important forage and fodder crop which is cultivated worldwide and harvested principally for hay used to feed cattle (Wiersema and León, 2012). Though *M. truncatula* is absent from the gene pool concept for *M. sativa*, it itself can be used as a forage crop and in soil improvement as well as having potential as a gene source for disease resistance in alfalfa (Wiersema and León, 2012).

With the recent release of sequence data for 3000 samples of rice (BGI and IRRI, 2014; www.gigasciencejournal.com/content/3/1/7), in comparison our study has a relatively small sample size, but focuses on a CWR rather than the crop species itself. Nevertheless it provides a demonstration of what can be achieved using sequence data to inform both the conservation and use of CWR. We have chosen *M. truncatula* for this demonstration because a reference sequence already exists. This presents an advantage over the alternative approaches where no such reference sequence is currently available, and where *de novo* sequencing or alternatively exome/RNA-Seq approaches would be required.

The sequencing has been undertaken using an Illumina HiSeq 2500 sequencing platform and has been facilitated by the new BGI-Birmingham Joint Centre for Environmental Omics at the University of Birmingham (www.birmingham.ac.uk/news/latest/2012/07/31-July-Beijing-Genomics-Institute-partners-with-the-University-of-Birmingham.aspx). Using the TruSeq V3 chemistry and the high output run mode the process can generate up to 300 Gb of data in a single flow cell, allowing close to 40x coverage of the 500 Mb genome of *M. truncatula*.

The seed accessions to be sequenced were obtained from the gene bank of the International Centre for Agricultural Research in the Dry Areas (ICARDA), and they originated from a wide geographical range (Australia, Azerbaijan, Czech Republic, Hungary, Italy, Jordan, Lebanon, Libya, Malta, Morocco, Portugal, Syria and Turkey).

Upon completion of the sequencing, Single Nucleotide Polymorphisms (SNPs) from across the genome will be selected. These will then be used to determine levels and patterns of genetic diversity distribution in association with the available ecogeographic information in order to inform conservation of this species, including genetically informed gap analysis for targeted collection. In addition, we have already selected over 50 genes known to be associated with biotic and abiotic stress tolerance in *Medicago*, and we expect to use SNP variation to identify adaptively useful variants of these genes which will be suitable by way of gene transfer for improvement of the *M. sativa* crop.



Line drawing of a *Medicago truncatula* plant (Source: Al-Atawneh *et al.*, 2009)

“With the costs of sequencing plummeting and data analysis tools advancing rapidly in their capability, it should be possible to sequence most/many CWR in the near future”

The ease with which we have been able to undertake this high-throughput sequencing is partly due to the existence of a reference genome sequence for *M. truncatula*. For a range of reasons, sequencing multiple genomes of other CWR will not necessarily be as easy, not least because assembly will need to take place against the backdrop of the actual crop species acting as the nearest reference genome, or in the absence of a reference genome, so requiring *de novo* sequencing. However, even for *Medicago* there are additional complex issues not unrelated to taxonomy, and particularly related to the use of the Harlan and De Wet Genepool classification system. According to one treatment, the species whose genome has been most convenient to sequence (due to its relatively small size (500 Mb), and it being diploid rather than polyploid), namely *M. truncatula*, does not even appear in the tertiary gene pool of *M. sativa*, the actual crop (Wiersema and León, 2012). Although Harlan and de Wet (1971) state that normally any species in the same genus as a crop would be in the crop's tertiary Genepool, the Wiersema and León (2012) conclusion does raise the issue as to how easy it will be to use the *M. truncatula* sequence for analysing other genomes of the *Medicago* genus.

Nevertheless, with the costs of sequencing plummeting and data analysis tools advancing rapidly in their capability, it should be possible to sequence most/many CWR in the near future with a view to enhancing both their conservation, through targeted collection, and ultimately their use.



Photograph of *Medicago truncatula* fruits (Source: Al-Atawneh *et al.*, 2009)

References

- Al-Atawneh, N., Shehadeh, A., Amri, A. and Maxted, N. (2009) *Conservation Field Guide to Medics of the Mediterranean Basin*. ICARDA, Syria. 214 pp.
- BGI and IRRI (2014) The 3000 rice genomes project. *GigaScience* 3(7).
- Dias, S., Dulloo, M.E. and Arnaud, E. (2012) The role of EURISCO in promoting use of agricultural biodiversity. In: Maxted, N., Dulloo, M.E., Ford-Lloyd, B.V., Frese, L., Iriondo, J.M. and Pinheiro de Carvalho, M.A.A. (eds.), *Agrobiodiversity Conservation: Securing the Diversity of Crop Wild Relatives and Landraces*. CABI Publishing, Wallingford. Pp. 270–277.
- Harlan, J.R. and de Wet, J.M.J. (1971) Towards a rational classification of cultivated plants. *Taxon* 20: 509–517.
- Wiersema, J.H. and León, B. (2012) GRIN Taxonomy crop wild relative project. USDA, ARS, National Genetic Resources Program. National Germplasm Resources Laboratory, Plant Exchange Office, Beltsville, Maryland. www.ars-grin.gov/cgi-bin/npgs/html/queries.pl

On the sustainable use and conservation of plant genetic resources in Europe

L. Frese¹, A. Palmé², L. Bülow¹, G. Neuhaus¹ and C. Kik³

¹Julius Kühn Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Breeding Research on Agricultural Crops, Erwin-Baur-Str. 27, D-06484 Quedlinburg, Germany.

²Nordic Genetic Resource Centre (NordGen), Box 41, SE-230-53 Alnarp, Sweden.

³Centre for Genetic Resources, The Netherlands (CGN), Wageningen University and Research Centre, Droevendaalsesteeg 1, NL-6708 PB Wageningen, The Netherlands. Email: chris.kik@wur.nl

There is a wealth of plant genetic resources (PGR) in Europe, both growing *in situ* and conserved in gene banks. The material is often well adapted to the local conditions and contains important traits that have a potential to facilitate adaptation of European crops to climate change and other future challenges. However, the use of PGR in Europe is far from optimal and many useful resources are un- or under-used. Therefore, a study was conducted in the framework of the EU-funded PGR Secure project to better understand the reason behind this and to suggest a way forward. Furthermore, a web-based platform for stakeholders specifically interested in

conservation and use of PGR has been developed to enhance the exchange of information and cooperation within this community.

Study on the European stakeholders in the conservation and use of plant genetic resources

To gather data for the study, around 120 PGR stakeholders from all over Europe were interviewed face-to-face (Fig. 1). In addition, an online questionnaire was distributed which was duly answered by 220 respondents (Table 1) and fully completed by 131 persons.



Figure 1 Data on the conservation and use of plant genetic resources was collected by interviewing stakeholders in the countries marked in red

Based on these data a preliminary SWOT (strengths, weaknesses, opportunities and threats) analysis was conducted, which was subsequently discussed at a three-day stakeholder workshop in 2013 (www.nordgen.org/index.php/en/content/view/full/2481/). Over 80 representatives from gene banks, breeding companies, public research institutes, agro-NGOs and government took part in the workshop (Fig. 2a). This was the first time that a diverse range of stakeholder groups had been brought together to debate a common concern—that of the current state of the plant germplasm system in Europe, from conservation to utilization of the full range of PGR that exists in the region. The workshop was organized in stakeholder specific working group sessions. The members of the working groups discussed the preliminary results of the SWOT analysis and the targets and strategies proposed by the PGR Secure team leading the work. The results of the working groups were reported during the final plenary session (Fig. 2b).

During the workshop, a stakeholder market day was organized in which representatives of the stakeholder groups had the opportunity to present their institute or organization and to establish new contacts (Fig. 2c). The stakeholder workshop was considered highly beneficial, particularly with regard to establishing a dialogue with the breeding research and plant breeding communities in terms of their priorities and needs.

Based on this workshop a final report entitled 'On the sustainable use and conservation of plant genetic resources in Europe' was published (www.pgrsecure.bham.ac.uk/sites/default/files/documents/public/D5.5_Report_on_PGRFA_use_in_Europe.pdf).

“In our vision of a European Plant Germplasm System the separate national plant germplasm systems are well interconnected with an efficient flow of information and material”

SWOT analysis: approach and results

The SWOT analysis was conducted using a formalized procedure including three steps: step 1 – identification of target states of a European Plant Germplasm System; step 2 – identification of strengths, weaknesses, opportunities and threats; and step 3 – development of strategies on how to reach the target states.

Both internal (strengths and weaknesses) and external factors (opportunities and threats) that affect the plant germplasm system in Europe were identified (Fig. 3). Each national plant germplasm system is composed of the conservation sector (ex

Table 1 Number of respondents by country and stakeholder group (B= plant breeder, Gov= government representative, PR= public breeding research institute, GB= gene bank, NGO= non-governmental organization). The questionnaire was started by 225 persons of whom five opted out after the first few questions.

EU Countries	B	Gov	PR	GB	NGO	Total	%
Netherlands	18	0	7	5	5	35	15.9
Germany	11	8	9	1	2	31	14.1
France	4	1	9	4	2	20	9.1
Sweden	6	1	6	2	1	16	7.3
Denmark	3	2	6	1	1	13	5.9
Spain	1	1	5	3	2	12	5.5
Italy	3	1	5	0	1	10	4.5
Norway	3	3	3	1	0	10	4.5
Bulgaria	0	2	4	2	1	9	4.1
Finland	1	3	3	0	1	8	3.6
Poland	1	1	5	1	0	8	3.6
Austria	1	1	2	2	1	7	3.2
Greece	1	1	2	2	1	7	3.2
Czech Republic	2	1	2	1	1	7	3.2
Latvia	1	0	4	1	0	6	2.7
Romania	1	0	4	1	0	6	2.7
Iceland	0	2	1	2	0	5	2.3
Slovenia	0	0	4	0	1	5	2.3
Estonia	0	1	1	1	0	3	1.4
Lithuania	0	1	0	1	0	2	0.9
Totals	57	30	82	31	20	220	100



Figure 2 (A) Participants of the stakeholder workshop in Wageningen, November 2013; (B) Final plenary workshop session; (C) Stakeholder market session to initiate new contacts

situ, *in situ* and on-farm; symbolized in the figure by the term gene bank), research institutes, plant breeders and their agricultural customers as well as agro-NGOs. The system is influenced by policy, legislation and authorities, reports on PGR in media, consumer decisions, as well as market developments. Internal factors are attributes of the institutions shown in the inner circle that can be exploited or minimized to achieve a target. The institution owns the attribute and controls it. External factors are developments or decisions that cannot be controlled by the institutions shown in the inner circle. External factors have positive or negative impacts on the relationship between and the functioning of the institutions. A SWOT analysis can be performed at the level of national plant germplasm systems and at the European level as well. In our vision of a European Plant Germplasm System the separate national plant germplasm systems are well interconnected with an efficient flow of information and material.

The workshop participants agreed on a series of targets to be achieved per stakeholder group. To illustrate the choice and formulation of a strategy, target state 1 of the gene bank stakeholder group is taken as example.

Step 1: Definition of a target

Target state 1: European countries interacting with the European Plant Germplasm System do so on the basis of a national biodiversity/agro-biodiversity strategy and a national PGR programme.

Step 2: SWOT of the stakeholder group 'gene bank'

Three items per SWOT category are presented here that were considered by the workshop participants as most important.

Strengths

1. Gene banks within the countries involved in the study generally manage collections that are in accordance with the requirements of the user community (public research, plant breeders and their agricultural customer as well as agro-NGOs).
2. All of the interviewed gene banks have established computerized gene bank information systems. Some of them operate web-based information systems allowing users to search for passport, characterization and evaluation data and to order accessions.
3. All countries provide some funds for conservation activities through national agrobiodiversity and other environmental protection strategies.

Weaknesses

1. Gene banks seldom lead the development of national *in situ* and on-farm management strategies and funding for *in situ* / on-farm programmes is often not constantly available. Many different governmental authorities are responsible and often a national strategy for *in situ* / on-farm management is missing.
2. Most gene banks are not independent with respect to their programme, funds or staff. Crop experts are thus forced to prioritize breeding research work at the expense of conservation work.
3. *Ex situ* management of PGR of many EU gene banks is not based on defined, written quality management standards such as the AEGIS Quality Management System (AQUAS). Limited funding exists for maintaining and regenerating PGR and consequently many accessions lack viability data. Insufficient funding impedes the identification of duplicates within and between the collections and the rationalization of gene bank holdings.

Opportunities

1. PGR conservation activities are backed up by policy through national agrobiodiversity and other nature protection strategies in most countries, and in some countries conservation measures are further detailed in national PGR expert programmes.

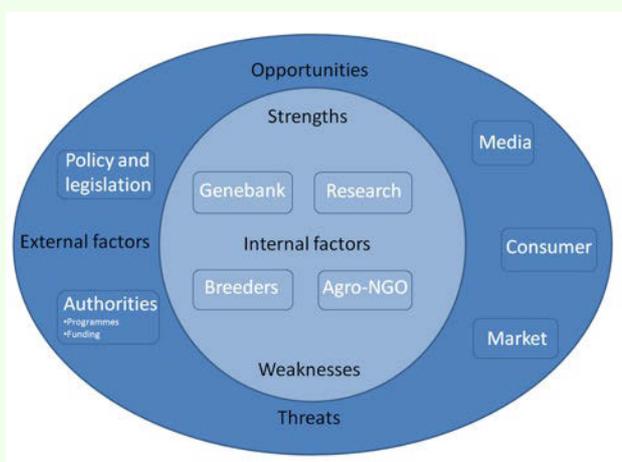


Figure 3 Factors affecting the plant germplasm system in Europe

2. EU programmes can support national *ex situ*, *in situ* and on-farm conservation activities. The EU Research and Innovation programme Horizon 2020 supports scientific progress and provides methods, data, information and knowledge which can be used to improve germplasm conservation actions. The agri-environmental measures of the Rural Development policy can facilitate the transfer of scientific innovation into agricultural practice.
3. EURISCO could potentially act as the information backbone of a European Plant Germplasm System and the goal should be one integrated European information system. European Central Crop Databases (ECCDBs) exist and demonstrate how characterization and evaluation data can be recorded according to the single observation concept and made available online.

Threats

1. Insufficient support of germplasm conservation activities at all governmental levels.
2. The European Cooperative Programme for Plant Genetic Resources (ECPGR) is currently renovated, however, without a substantial increase of the budget. If the programme continues to rely on input in kind by the stakeholder community, it may not longer be supported by the community. This would result in the loss of the only body in Europe experienced in the coordination of PGR activities.
3. The native plant species and habitat conservation sector and the agricultural PGR conservation sector do not cooperate in the field of *in situ* conservation in a structured way.

Step 3: Choice of formulation of a strategy

Different types of strategies can be derived from a SWOT analysis (Table 2). The strategy thought to be best suited to improve the analysed system is then applied.

Table 2 Different types of strategies that can be derived from a SWOT analysis

Strategy	Abbreviation	Explanation
Strength and opportunity	SO	Use strengths to take advantage of opportunities
Strength and threat	ST	Use strengths to reduce the likelihood and impact of threats
Weakness and opportunity	WO	Make use of opportunities to overcome weaknesses
Weakness and threat	WT	Minimise weaknesses and threats

For example, in the gene bank SWOT analysis, strength number 3 and opportunity number 2 are combined and used to formulate an SO strategy as follows: Use existing strategies and PGR national work programmes as blueprints for further development of national biodiversity/agro-biodiversity strategies and expert programmes as well as a matching EU strategy and work programmes. Use arguments provided by EU policy documents such as 'Our life insurance, our natural capital: an EU biodiversity strategy to 2020' of the Council of the European Countries (Commission document SEC(2011) 540 final) to underpin the need for a well-organized European Plant Germplasm System.

Main results of the study

The main outcomes from this study as published in the final report are twelve recommended actions aimed at improving the conservation and use of plant genetic resources in Europe:

1. The EU should consider the establishment of a sufficiently funded organizational and technical European infrastructure for conservation and use of plant genetic resources, which integrates existing national components and assists in the enhancement of these national components.
2. This infrastructure should be developed by the member states towards a European Plant Germplasm System whereby it should be noted that the separate national units are well interconnected to guarantee an efficient flow of information and material among them.
3. The CBD and ITPGRFA, as well as derived policy papers, should be used by the member states and stakeholder groups to establish a legal basis for conservation of plant genetic resources for food and agriculture in the EU.
4. Gene banks are often an integral part of a research institute and seldom independent in terms of mandate, programme, staff and budget. The member states should take a status change of gene banks towards juridical and economic independence into consideration.
5. The gene banks should describe their financial needs and explain why additional funds are needed and how funds would be invested to secure existing gene banks and to develop them towards a component of a European Plant Germplasm System.
6. The establishment of a comprehensive European information infrastructure for plant genetic resources should be politically and financially supported by the European Commission.
7. The visibility and access to, and functioning of national plant germplasm systems should be improved by the member states to facilitate the broader use of genetic resources in crop genetic enhancement programmes.
8. National and EU authorities should clear uncertainties concerning access and benefit sharing (ABS) rules so that stakeholders can take decisions on a safe legal basis.
9. The European agro-NGOs and their influence should be strengthened.
10. A European network of Private-Public-Partnership projects should be established by the stakeholder groups and supported by the European Commission, which also includes less competitive crops that play a significant role for broadening the interspecific diversity in European crop production systems and thus contribute to food security.
11. Research performed within national plant germplasm systems (see Fig. 3) in Europe should guarantee a sufficient understanding of the amount and distribution of genetic diversity present in priority crop gene pools.
12. Breeding experts and policymakers should together develop a European priority list of long-term crop specific pre-breeding programmes deemed necessary to cope with challenges for the plant breeding sector arising in the future.

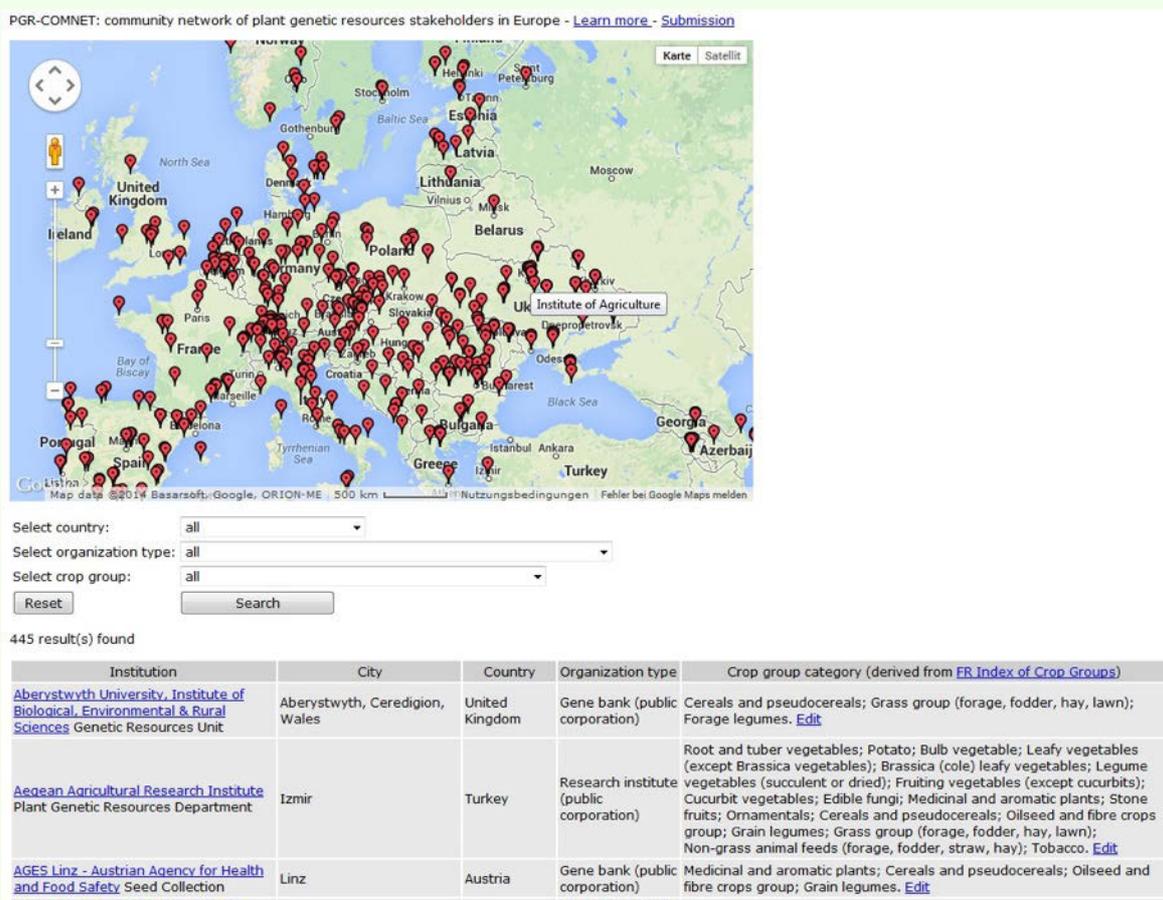


Figure 4 PGR-COMNET, map and table displaying European institutions involved in PGR conservation and use. PGR-COMNET is linked with the ECPGR and EUCARPIA websites, and with Arca-Net, a web-based platform of the agro-NGO sector.

Development of a European plant genetic resources conservation and use 'who is who' platform

Members of the ECPGR can easily be found via the ECPGR website and the lists of working group members. However, researchers from academia and industry or persons working in the agro-NGO sector are rarely represented on the ECPGR website. Cooperation between the conservation and user sectors can be improved by visualizing the location of institutions on a European map and by describing their specific interest.

At European level, there are several instruments for researchers from academia and industry to establish partnerships for joint research projects. However, a platform for stakeholders specifically interested in conservation and use of PGR has not been established so far. Therefore, the PGR-COMNET has been created within the framework of the PGR Secure project and has been designed as a community network of and for European institutions working specifically in the field of PGR conservation and use. PGR-COMNET (<http://pgr-secure.jki.bund.de>) displays the institutions on a map and additionally provides information on the crops the institutions are interested in within a separate table (Fig. 4).

By giving an overview on European actors interested in plant genetic resources, PGR-COMNET aims to promote contacts and partnering among the different institutions. Basic search functionality enables queries for specific countries, for selected stakeholder groups or certain crop groups. A user-friendly online submission tool allows users to create new or update ex-

“By giving an overview on European actors interested in plant genetic resources, PGR-COMNET aims to promote contacts and partnering among the different institutions.”

isting entries. PGR-COMNET's sources of information have so far been the online questionnaire conducted in the course of the PGR Secure project, the EURISCO list of European gene banks provided by Bioversity International, and individual submissions by institutions. For higher visibility, a site at www.pgrsecure.org/pgr-comnet embeds PGR-COMNET within the framework of the PGR Secure project homepage. It currently harbours 445 institutions from 44 different European countries. PGR-COMNET's content will be expanded and updated on a regular basis also beyond the PGR Secure project lifetime.

Acknowledgements

This project was funded by a grant from the EU 7th Framework Programme for the PGR Secure project (Grant agreement no. 266394), and a grant from the Ministry of Economic Affairs of the Netherlands (project no. WOT-03-002-040) for the contribution of CGN.