ECPGR Activity Grant Scheme Proposal Form

Fifth Call

Activity Proposal

Activity	
Full title	Testing, Use and Alignment of genetic data to distinguish unique and characterized accessions in Prunus
Acronym (or short title)	Prunus Alignment
Duration of Activity (in months)	24
Start date – End date Please indicate start date not earlier than 3 months after deadline of call	1st February 2018 – 31st January 2020

Applying Working Group(s)

	Working Group	Indicate name and surname of Working Group Chair
1.	*Prunus*	Daniela Giovannini
2.		
3.		
4.		

Activity Coordinator

Activity Coordinator	
Name and Surname	Dr Matthew Ordidge
Nationality	UK
Current position	Research fellow, and Scientific Curator of UK National Fruit Collection
Institute	University of Reading
Country	UK
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Activity Partners

Please note that each partner needs to be a member of a Working Group's Pool of Experts to be eligible.
A maximum of 12 funded partners can be listed. For self-funded partners please use the separate box below.

Partner ID No.	Name and Surname	Institute	Country
1	Daniela Giovannini	CREA-OFA, Forli	Italy
2	Gunars Lacis	Institute of Horticulture, Latvia University of Agriculture	Latvia
3	Marine Delmas	INRA	France
4	Monika Hofer	Julius Kuhn-Institute, Institute for Breeding Research on Fruit Crops	Germany
5	Pavlina Drogoudi	Hellenic Agricultural Organization 'Demeter'	Greece
6	Stein Harald Hjeltnes	Njoes naeringsutvikling	Norway
7	Hedi Kaldmae	Estonian University of Life Sciences, Polli Horticultural Research Centre	Estonia
8	Matthew Ordidge	University of Reading	UK
9	Torben Bo Toldam-Andersen	University of Copenhagen	Denmark
10	Zsuzsanna Bekefi	National Agricultural Research and Innovation Centre, Fruitresearch Institute	Hungary
11	Felicidad Fernandez	NIAB EMR	UK
12	Gabriella Sonnante (representing Giovanni Vendramin)	Institute of Biosciences and Bioresources (IBBR-CNR)	Italy
13	Hilde Nybom	Department of Plant Breeding, SLU	Sweden

Self-funded partners

Partner No.	Name and Surname	Institute	Country
1	Dunja Kazija	Institute of Pomology, Croatian Centre for Agriculture, Food and Rural Affairs	Croatia
2	Marc Lateur	Agricultural Research Centre (CRA-W)	Belgium
3	Frantisek Paprstein	Research and Breeding Institute of Pomology Holovousy Ltd	Czech Republic
4	Jiri Sedlak	Research and Breeding Institute of Pomology Holovousy Ltd	Czech Republic
5			
6			

Description of Activity (suggested max. 1000 words)

Please address the following aspects:

– Background: Explain the context behind the choice of this Activity, e.g. why this has been prioritized or selected. If this is the continuation of a preceding Activity, please indicate how and why the new Activity will build on previous results/experiences.

Previous projects (PRUNDOC and EUCHERRY) have allowed the genotyping of sets of plum (38) and sweet cherry (212) accessions to identify unique material for inclusion within AEGIS. These accessions have also been characterised using common and harmonized first priority descriptors (FPD). Data were submitted to the Prunus DB manager and prepared for inclusion in EURISCO. A complementary project within the Malus/Pyrus WG is building upon equivalent background to utilise existing data, networks, and expertise in bringing together genotypic (SSR) data from multiple national sources to expand on the ECPGR European data base.

PRUNDOC identified, and characterized (with FPD) 57 further accessions which were unable to be genotyped (due to funding limitations) and classified these as, in principle MAA's, subject to a lack of duplication to be tested by subsequent genotyping; 39 of these are held by partners in this proposal.

EUCHERRY additionally widened the SSR data coverage through the inclusion of a series of 112 accessions submitted (and co-funded) through a COST proposal.

Furthermore, a recent study published by PRUNDOC member Pavlina Drogoudi and colleagues has highlighted a potential technique to speed up and improve the efficiency of genotyping in plums.

These previous efforts provide a base on which to further develop our understanding of ECPGR Prunus germplasm and further improve the representation of Prunus within AEGIS and data within EURISCO.

– Justification: Explain why this Activity is justified in terms of making progress towards achieving the ECPGR objectives.

Confidence in the genetic uniqueness of material is fundamental to the development of the AEGIS European collection. It is clear that SSR markers are a useful tool in plums (as with many crops) but the hexaploid nature of Prunus domestica means that scoring SSR markers is expected to be challenging (and has consequently been limited in many collections so far). A consideration of new opportunities to approach the genetic distinction of accessions in a more efficient manner will be valuable for genebank curators within ECPGR.

The alignment of genotyping data from existing resources provides an opportunity to both increase the coverage of genotypic data and to make the data more available to curators and researchers in a wider range of countries. Building upon an existing ECPGR supported effort and working in line with complementary activities carried out by the Malus/Pyrus group will allow a more consistent approach to be taken within the fruit crops under ECPGR.

– Rationale for the choice of partners: Explain why the selected partners are the most suitable to carry out the proposed Activity and briefly describe their respective roles in the Activity.

The expertise and experience of SLU (partner 13) was integral to the SSR genotyping of plums in PRUNDOC and we would expect that their inclusion as a partner was crucial to align with this previous effort.

Pavlina Drogoudi (partner 5) was a partner in PRUNDOC (and self-funded partner in EUCHERRY)

and was involved in the previous development and testing of the HRM technique for genotyping plums.

NIAB-EMR (partner 11) provided a genotyping service and expertise in EUCHERRY and we would expect that their inclusion in this project, to fingerprint some overlapping accessions, would be essential to allow the best alignment of datasets with the EUCHERRY dataset.

Partners 1-6 submitted samples for genotyping in PRUNDOC and partners 1-5 have committed to supplying second priority descriptor (SPD) data for these accessions (partner 6 is unable due to regeneration activity).

Partners 1-6 are able to offer samples from accessions identified, but not previously genotyped, in PRUNDOC for analysis, and have committed to supplying SPD for these accessions (noting the above for partner 6).

Partners 7-10 are able to offer novel plum germplasm to expand upon the PRUNDOC set and are able to commit to supplying first priority descriptor data and leaf samples for genotyping to the project.

Partners 1,2,8 and 12 are able to offer testing capability for the analysis of the HRM technique across multiple labs.

Partners 1, 4, 8 and 13 are able to supply existing cherry SSR data screened with recommended ECPGR (or EUCHERRY) markers for alignment and inclusion in the ECPGR Prunus DB.

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*– **Methodology or Approach:*** Explain how the partners will operate. Clearly explain who is expected to do what. Also explain the rationale of meeting (or not) as part of the Activity. Include a Gantt Chart, to illustrate the work breakdown structure of the project.

Leaf samples from the 39 accessions identified, but not previously genotyped, in PRUNDOC will be supplied for genetic analysis by partners 1-6; SPD will be collected for all unique PRUNDOC accessions by partners 1-5 (55 accessions characterized with SPD: 39 accessions genotyped).

Further plum samples will be supplied for SSR analysis and FPD data will be collected to expand on the PRUNDOC set by partners 7-10. It is expected that 10-12 accessions per partner will be characterized although budget limitations mean that only 5 per partner will be genotyped (48 new accessions characterized: 20 of which genotyped).

The completion of SSR fingerprinting of PRUNDOC accessions will be carried out by partner 13 along with the genotyping of the newly proposed accessions.

Attempts at comparing a subset of diverse SSR genotyped plum material from the PRUNDOC and/or new accessions will be made using HRM by partners 1, 2, 8 and 12 (with the help of collaborative input from Marco Pietrella at the organization of partner 1) to allow the comparison of findings; approximately 24 accessions will be replicated by each of partners 1, 2, 8 and 12 and compared to a dataset created for approximately 60 accessions genotyped using HRM by partner 5 (with a collaborative input of expertise by Dr Ioannis Ganopoulos of the same organization).

Pre-existing data for cherry will be supplied by partners 1 (~150 accessions), 4 (~100 accessions), 8 (~300 accessions) and 13 (~20 cultivars) and additional cherry data are expected to be supplied in collaboration by Stephanie Mariette (INRA, France) [~200 accessions] and Markus Kellerhals (Agroscope, Switzerland) [national dataset accession number unknown].

A subset of approximately 140 accessions from across the collections with pre-existing data will be genotyped in concert by partner 11 and data will be aligned to allow the alignment of collections.

Plum SSR data will be collated by partner 13 and submitted to the ECPGR Prunus database such that it will be available to EURISCO when capability is developed.

A comparison of HRM methods for plum across different labs will be made by partners 1,2,5,8 and 12 for discussion with the remaining members at the project meeting.

Cherry SSR data will be aligned by partner 11 and submitted to the ECPGR Prunus database such that it will be available to EURISCO when capability is developed.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Plum SSR genotyping					▓	▓	▓																	
Plum F/SPD Characterization		▓	▓	▓	▓	▓	▓	▓	▓	▓	▓	▓	▓	▓	▓	▓	▓	▓	▓					
HRM assessment on subset of plum								▓	▓	▓	▓	▓	▓	▓	▓	▓	▓	▓	▓					
Cherry SSR genotyping for alignment					▓	▓	▓	▓	▓	▓	▓	▓	▓	▓	▓	▓	▓							
Project meeting																					▓	▓		
Project report completion																					▓	▓	▓	▓

– Description of genetic material: If your Activity is focusing on genetic material, please describe in detail, as far as possible, who is providing this genetic material, its status and the number of accessions under investigation (for example: *This Activity aims at molecularly analyse/safety-duplicate/evaluate/collect XY accessions (listed) of "Genus species", provided by genebank Z/ farmers in country W /to be collected in country P…, etc.*).

SSR genotyping will be carried out on the PRUNDOC accessions which were not fingerprinted previously. In addition, a subset of newly proposed accessions will be screened for uniqueness. HRM will be assessed on a selected subset of these accessions.

New plum material will be supplied by partners 7-10.

– Expected impact. Clearly specify the expected impact from this Activity for the respective ECPGR objective(s), compared to the current state of progress of those same objectives. Explain how the impact will be obtained.

The project will instantly confirm, by genotyping a series of plum accessions suitable for inclusion in AEGIS (for the majority of which, first and second priority descriptor data will be available). It will also identify a further set of 40-50 plum accessions with FPD for subsequent inclusion in AEGIS and a subset of approximately 20 of these will be checked for uniqueness by genotyping.

Recommendations on future plum genotyping techniques will be made to improve the understanding of members.

A further expanded cherry SSR dataset will be produced to allow a wider comparison of material for the identification of further unique accessions for inclusion in AEGIS in the future.

– Links with other non-ECPGR projects or individuals: If applicable, clearly explain the objectives of the linked projects and the reasons for complementarity with the ECPGR Activity.

As part of the Prunus group's ongoing collaborative 'in kind' efforts, characterization of the whole set of PRUNDOC proposed accessions (i.e. those previously genotyped, and those not) will be completed

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using second priority descriptors by the project members (with the exception of member 6). The inclusion of these descriptive data will further strengthen the inclusion of the full set of accessions in AEGIS following the proposed genotyping in this project.

As part of the nationally funded curation of the UK National Fruit Collection, a set of SSR data will be being produced for cherry during 2017/18. These data will be made available to the project, and consequently ECPGR members to expand upon the coverage of data available for use in curation and research. Equivalent datasets will be made available by: partner 1, who will provide data produced as part of a national assessment of local and regional collections in Italy; partner 4, who will provide data from the molecular evaluation of the German Fruit Genebank and partner 13 who will provide data from the analysis of accessions at SLU, Sweden.

Additional collaborative agreements have been made with Stephanie Mariette (INRA, France) and Markus Kellerhals (Agroscope, Switzerland) to attempt to align further SSR data for cherry, again produced under national projects.

Expected products and related ECPGR Objectives

List concrete products and results that are obtained by the Activity and the corresponding number(s) of the ECPGR Outcome(s) and/or Output(s) and/or Activities to which each product/result will contribute.

	Expected products/results	Corresponding ECPGR outcome, output, activity
1	Completed SSR genotyping of PRUNDOC prioritised accessions and additional accessions nominated by new members – data submitted to ECPGR Prunus DB and EURISCO (when capability is developed)	All accessions identified in PRUNDOC and some new material will be genotyped and characterized ready for inclusion in AEGIS where appropriate.
2	Comparison and recommendations on the use of HRM techniques for the identification and discrimination of plum accessions within ECPGR based on previously and/or newly SSR genotyped material.	Recommendations for the future coordinated genotyping of plums will be documented though publication in a scientific paper.
3	Alignment of EUCHERRY SSR data with existing data generated from national collections submitted for inclusion in the ECPGR Prunus DB.	An expanded SSR dataset will allow the identification of further unique material for inclusion in AEGIS in the future.

Workplan for the proposed period of the Activity

Brief description of meetings and/or main actions of the Activity.

	Type of Action *(indicate if "meeting" or "other action")*
1	Genotyping of plum for the completion of the PRUNDOC MAA's and assessment of new material - June-August 2018
2	Characterization of new plum accessions using agreed first priority descriptors and completion of second priority descriptors for PRUNDOC material held by partners 1-5 – March 2017-September 2019
3	Assessment of alternative (more efficient) techniques for future co-ordinated genotyping in plum – September 2018-August 2019
4	Data alignment of existing datasets against the EUCHERRY database – June 2018-August 2019
5	Meeting to discuss and disseminate the findings of the above - September/October 2019

Additional remarks

Indicate any additional remark(s) that is/are important for the evaluation/implementation of the proposed Activity

Remarks:

**Please send the completed form together with the budget table to the
Chair of the submitting Working Group for submission of the Activity proposal.**