Meeting of the ECPGR Activity 'Prunus Alignment'

18 February 2020, Athens, Greece

REPORT OF THE MEETING

Opening of the meeting and welcome

Dr Eirini Pittara, General Director, General Directorate of Agricultural Research, HAO-Demeter being unfortunately unable to attend the meeting, Pavlina Drogoudi welcomed the participants on behalf of HAO-Demeter and gave an initial presentation[1] on the role of the organization. HAO-Demeter acts as the national body for agricultural research and technology in Greece. The organization also acts in the control of supply of products as well as the protection of product origin identity. The organization additionally carries out vocational training and encompasses 11 research institutes. Basic research priorities focus on climate change, issues around the Nagoya Protocol and the bioeconomy. Of particular relevance to the group are the Institute of Plant Breeding and Genetic Resources in Thessaloniki and within that, the Department of Deciduous Fruit Trees, located in Naoussa.

Genotyping (by conventional SSR) of PRUNDOC and new samples (including the collection of characterization data) *(M. Ordidge and H. Nybom)*

Molecular markers in Plums – a summary of the progress in Prunus Alignment

H. Nybom

A summary of the available molecular marker types (AFLP, SSR, SNP, DArT and Sequence-based) and opportunities for genotyping in *Prunus* crops was presented. The group discussed the potential and limitations of the current technologies. In summary, it was noted that SSR possibly remained the marker technology of choice due to the flexibility that it offered to genebanks and the genetic resource community (given the ability to run small numbers of samples in a basic molecular lab, sufficiency of data for identification and relatively low requirement for bioinformatic support). It was noted that an issue was expected to arise in the future around the support for capillary sequencing equipment and that older machines were already beginning to be phased out. The group acknowledged that this was an issue, but that in many cases it was expected to remain possible to use service providers to analyse samples (and this was already the mode of operation for some partners). SNP and potentially Sequence-based technologies offered the opportunity to generate considerably more data but were currently only efficient within large projects and were not expected to be as accessible to members as the SSR technology. It was also noted that significant value remained in the datasets that had been amalgamated, and the potential alignment of them, as was the aim of Prunus Alignment.

[1] All presentations will be available online (see here)

The recently published study combining Prunus Alignment results with those from PRUNDOC[2] was summarized. It was acknowledged that Fuad Gaši (Faculty of Agriculture and Food Sciences, University of Sarajevo, Sarajevo, Bosnia and Herzegovina, Member of the ECPGR *Malus*/*Pyrus* Working Group) had led much of the statistical analysis as a collaboration with the *Prunus* Working Group members. The study had used a smaller number of markers than are used in, for example *Malus*/*Pyrus*, but since most of the material was hexaploid, the smaller number of markers revealed a large amount of information, nonetheless. The markers had revealed between 17 and 47 alleles per locus and this had resulted in between 30-35 alleles per genotype. There was some difficulty in calling alleles, due to the hexaploid nature of *Prunus domestica* and consequently markers had been run in simplex format. It was noted that there were relatively few loci for which six alleles were reported and that it would need to be accepted that occasional alleles might be missing from the dataset; the comparison of profiles should allow for occasional disagreement based on presence/absence. In the current dataset, most of the profiles had been run twice in order to aid with allele calling.

Twenty-three genotypes had been scored with a maximum of five alleles for any locus and a single sample was scored to have a maximum of two alleles for all eight loci (which was suggested to be worthy of further investigation in order to question either ploidy or species identification).

A number of accessions had come out as matching (see presentation) and these were discussed. Stein Harald Hjeltnes queried a finding relating to an accession by the name of 'Reine Claude Souffriau' and it was suggested that the source of this (Belgian) accession be investigated further.

A concern was expressed about the inconsistent use of molecular markers across different studies and it was agreed that the ECPGR study presented an opportunity to recommend a standard set of markers and reference cultivars to be used by ECPGR members (**Action 1**).[3] The group also discussed that morphological information (largely from characterization data) was broadly able to distinguish the main 'types' of plum, but was relatively uninformative in considering genetic diversity and that there were technical difficulties in comparing some of the data, since it appeared that different partners had interpreted standard scales differently. For example, an inconsistency in the use of intermediate values between countries and an inconsistency in the scale of characters such as overcolour was reported; it was noted that characters such as overcolour would be expected to be environmentally, and thus geographically variable.

It was reported that the small-fruited accessions appeared to hold more allelic diversity than the large-fruited accessions, potentially as a result of larger-fruited cultivars having been more widely distributed as clonal material from which seedlings have subsequently been selected. It was discussed that there remain significant numbers of (generally small-fruited) semi-wild accessions of plum in local collections and that these might represent an interesting focus for further investigation of diversity.

It was discussed that it had not been possible to detect any clear and meaningful geographic separation of genetic diversity, and again this appeared to point towards the fact that many of the larger-fruited selections will have been distributed clonally through recent history. It was

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[2] Gaši F, Sehic J, Grahic J. et al. 2020. Genetic assessment of the pomological classification of plum *Prunus* domestica L. accessions sampled across Europe. Genetic Resources and Crop Evolution. https://doi.org/10.1007/s10722--020-00901-y

[3] Agreed actions are listed in a table at the end of present report.

noted that there was a possibility of sampling bias caused by the selection of nationally representative accessions, often considered as ‘emblematic’.

It was questioned whether the raw genotype data were included in the publication and Hilde Nybom and Matthew Ordidge agreed that they would confirm and look into any issues that arose from this. The objective was that all data should be made available to the ECPGR *Prunus* Database (EPDB) and in turn EURISCO, potentially in a citeable format through placement in a public data repository if necessary (**Action 2**).

The group went on to discuss the overall project dataset, including characterization data and some discussion was held regarding the EPDB and its relationship with EURISCO. It was highlighted that EURISCO still lacks the ability to hold some of the data held by EPDB, and that it has not yet been fully clear that all descriptive data (from previous *Prunus* group projects) have been placed within the EPDB and in turn EURISCO. The group agreed to confirm the situation with respect to both First and Second Priority Descriptor data and also genetic marker data with a view that these are all made available either through the EPDB or other means. The concept of placing things in citable repositories was discussed and Matthew Ordidge agreed to follow this up within the project and to check with all members that all characterization data had been completed and made available (**Action 3**).

The group was updated that whilst the current hosting of the EPDB at INRA was unsustainable, an alternative hosting option within an active system at INRA had been proposed and would retain viability for the foreseeable future.

Genotyping of plum samples using the SSR-HRM technique
(M. Ordidge and I. Ganopoulos)

SSR analysis of plum accessions using High Resolution Melting

Ioannis Ganopoulos (Matthew Ordidge)

A summary of the objectives and process of using High Resolution Melting (HRM) analysis as a technique to screen SSR data in plums was presented by Matthew Ordidge on behalf of Ioannis Ganopoulos (who was unfortunately unable to attend the meeting).

The four partners attempting to produce HRM data for comparison then each reflected on their experiences:

Matthew Ordidge

The experience in the UK was that initial analysis appeared promising, although unfortunately, the HRM machine at the University of Reading had suffered a critical failure during the experimental run and it had consequently not been possible to produce a final dataset for comparison. It was not expected that the machinery would be repaired during the time of the project and so data from the UK replication were not expected to be available.

Gabriella Sonnante

Prior experience with HRM at IBBR-CNR had been in assessing SNP polymorphism within mapping populations of diploid species, where polymorphism was both relatively simple and known. In this analysis 36 samples had been analysed in the labs at IBBR-CNR. Detailed meta-analysis was awaited from the Greek partner, but the initial impression was that there was large variation between samples and this was not initially easy to interpret. Melting

temperature curves had been compared and these appeared broadly similar with a slight shift between samples. It was surprising to only see a single melting curve peak for each sample.

Marco Pietrella

Analysis at CREA-OFA had used a different machine to the Rotorgene that was originally used by the Greek partner. Preliminary analysis suggested that the melting temperature curves again appeared to be broadly similar although that there appeared to be some variability between replicates. As with the analysis by IBBR-CNR, in general a single melt curve peak was seen, although in a single case two peaks had been visible. Two odd results had been seen with the software reporting melting temperatures around 90˚C. An initial attempt to analyse the data using the machine software appeared to classify samples into variant groups, although only five groups were identified and in one occasion a replicated sample was called as two different variants (see presentation).

Gunārs Lācis

The analysis had been found to be an interesting exercise, the partner having only previously had experience of using HRM for single SNP calling. A number of similar issues to the other partners had been experienced. The partners were currently struggling with a data compatibility issue caused by an apparent lack of compatibility between the data output of the more recent (QIAGEN) version of the Rotorgene and the original Corbett machine.

Unfortunately, due to illness, Ioannis Ganopoulos was not able to advise on these issues during the meeting and it was agreed that a list of queries would be compiled (**Action 4**) to be presented to the Greek partner for consideration with the view of discussion via a Skype meeting.

Overall, based on preliminary analysis the partners felt that the analysis and comparison of profiles did not appear straightforward although the group acknowledged that the more complete and detailed meta-analysis was yet to be carried out and was expected to benefit greatly from the expertise and experience of the Greek partner.

It was questioned whether the expectation would be to generate a database of profiles and whether a potential switch to a HRM-based technique of genotype calling would limit any downstream analysis, such as that of parentage (which has been carried out recently in a number of studies on germplasm collections using conventional capillary-based SSR techniques). Matthew Ordidge stated that such a database would presumably be required if profiles were to be compared in the future and that, as far as he understood, it might limit this additional value, although the analysis of parentage remained secondary to the primary analysis of duplication and similarity for the purposes of identifying material for AEGIS. Nonetheless, this should be taken into consideration in any recommendations of how to proceed.

Alignment of EU.CHERRY and National SSR datasets for cherry *(M. Ordidge and F. Fernandez)*

ECPGR Prunus Alignment Project Update

Felicidad Fernández-Fernández

The work of Suzanne Litthauer who had carried out the laboratory analysis of samples at NIAB-EMR was summarized. It was noted that in carrying out the analysis, some improvements on allele calling had been made by Suzanne and these had also been used to update the original

EU.CHERRY dataset. The data for the Prunus Alignment project had only been released shortly before the meeting and preliminary analysis was underway. A number of initial potential duplications were identified based on exact matches in the data.

Matthew Ordidge then presented an initial consideration of the data in raw spreadsheet format. It appeared that the data broadly aligned with a consistent adjustment factor, although, against this background it was also clear that a small number of samples stood out as being in need of further investigation (potential collecting labelling or handling errors). Two examples were highlighted, where initial analysis appeared to identify a small set of errors in the UK national data as well as a set of 14 apparent errors in the EU.CHERRY data for German samples. In each of these cases, accessions previously submitted for EU.CHERRY had been re-analysed within Prunus Alignment and the re-analysis had helped to identify where the error in the data lay (although identifying the cause to be collecting, labelling or handing would require further investigation and potentially remain unfathomable). Matthew Ordidge urged a note of caution in that, whilst he expected that an aligned dataset should be possible to produce, it would inevitably continue to contain some error, since it had not been feasible to carry out either the EU.CHERRY or the Prunus Alignment analysis in replicate and any interpretation of findings should be made with this in mind. It was noted that a lack of true replication is standard in the majority of national, and scientifically published, datasets of SSR analysis within fruit tree collections. This is generally accepted as a pragmatic compromise in the absence of unlimited funding; any relationships that stand out as unexplainable when morphology and provenance are also considered should be interrogated further.

Some discussion followed around the potential strategy of publishing the project findings and it was generally considered that this should be done in a way that complemented any ongoing work on the prior EU.CHERRY dataset. It was agreed that collaborators at INRA would be approached for discussion since they had volunteered to take the lead on a publication from the joint EU.CHERRY and COSTFA 1104 data (**Action 5**).

The next steps *(M. Ordidge and D. Giovannini)*

Prunus WG - ECPGR objectives for Phase X and next steps

Daniela Giovannini

A summary of recent *Prunus* WG projects (PRUNDOC, EU.CHERRY) was presented and it was noted that whilst these projects had been carried out with an aim to support the inclusion of *Prunus* material within AEGIS there remained extremely few *Prunus* accessions nominated as AEGIS accessions. Daniela noted that it was imperative that the individuals within the group follow this up with their National Coordinators (**Action 6**). It was noted that there often remained a disconnect between the identification of material potentially fit for inclusion in AEGIS and the actual nomination of accessions by the National Coordinator (which was in many cases beyond the responsibility of individual members).

A number of opportunities were highlighted for the *Prunus* group to consider pursuing in the next rounds of ECPGR funding and members were encouraged to consider leading on the development of a proposal. Potential areas for work included investigations into the assessment of phytosanitary status, since this remained a challenge for the field-based collections within the AEGIS system. It was noted that in the absence of either national or international quarantine facilities there remained the likelihood that individual accessions might potentially enter AEGIS, only to have to be removed in the instance of becoming unavailable

due to quarantine restrictions (which might themselves relate either to the accession itself or the collection/site in which it resides). Hedi Kaldmäe noted that a number of actions relevant to this issue were proposed at the ECPGR AEGIS Workshop in Madrid (2018) although no further action had been reported.

It was discussed that a better understanding and capability for virus testing would be useful to the group and that this currently appeared to be an area of expertise that was lacking. However, the testing of individual accessions was discussed and was initially felt likely to be too expensive per accession to be something that could be addressed through the ECPGR funding system. Monika Höfer suggested that a potential meeting to compare phytosanitary practices might be useful for the group.

The potential for the application of the principles of PRUNDOC and EU.CHERRY to a wider selection of *Prunus* was discussed. Primary candidates were considered to be sour-cherry, almond and apricot and it was discussed that a combination of two of these might allow a project to remain more Europe-wide since there was a tendency for each of them to be associated within limited regions.

Torben Bo Toldam-Andersen suggested that there appeared a need to carry out further work on standardizing morphological assessment and also noted that there was potentially valuable work that could be done to engage with local experts in pomology as the expertise in this area was being lost. It was noted that a balance would need to be met on where pomological knowledge could potentially be applied to the use of material for breeding.

Daniela Giovannini suggested that one of the most important issues was to make sure that listings (both EPDB and EURISCO) were as up-to-date as possible.

The following actions were agreed:

Action #		**Responsible**	**Deadline**
1	Consider the plum SSR marker set and samples included within the Prunus Alignment and PRUNDOC analysis, as a basis on which to recommend a standard set of markers and genotypes for SSR analysis of plum germplasm within ECPGR (including consideration of whether to communicate any recommendation through the project report and/or through a scientific publication [likely Acta Hort]).	Hilde Nybom, Matthew Ordidge	Consideration with respect to project report – 30 April 2020 Consideration w.r.t scientific publication – 31 Dec. 2020
2	Confirm the data availability of plum SSR data and submit to EPDB and, in turn, EURISCO (including consideration of whether to place data into a citable repository).	Matthew Ordidge, Hilde Nybom	30 April 2020

Action #		Responsible	Deadline
3	Confirm the availability and submission of project characterization data to EPDB and, in turn EURISCO (including consideration of any need to place data in a citable repository).	Matthew Ordidge	30 April 2020
4	Compile a list of queries and comments regarding partners' experience of HRM to be discussed with the Greek partner.	Gabriella Sonnante, Marco Pietrella, Gunārs Lācis, Matthew Ordidge, Ioannis Ganopoulos	13 March 2020
5	Approach colleagues at INRA regarding progress towards publication of the EU.CHERRY (and COSTFA 1104) data.	Felicidad Fernández-Fernández, Marine Delmas, Matthew Ordidge	31 March 2020
6	Consult National Coordinators on the nomination of 'potential' AEGIS candidates for formal inclusion.	All partners	31 July 2020

Annex 1. Agenda

Venue: AirHotel Parthenon, Athens, Greece

Tuesday, 18 February 2020

9:00	Welcome by Pavlina Drogoudi, on behalf of Dr Eirini Pittara, General Director, General Directorate of Agricultural Research, HAO-Demeter
9:15	Genotyping (by conventional SSR) of PRUNDOC and new samples (including the collection of characterization data) *(M. Ordidge and H. Nybom)*
10:30	*Coffee break*
11:00	Genotyping of plum samples using the SSR-HRM technique *(M. Ordidge and I. Ganopoulos)*
13:00 – 14:00	*Light lunch*
14:00	Alignment of EUCHERRY and National SSR datasets for cherry *(M. Ordidge and F. Fernandez)*
15:30	*Coffee break*
16:00	The next steps *(M. Ordidge and D. Giovannini)*
20:00	*Social dinner*

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