PhD School on Agriculture, Environment and Bioenergy
(http://sites.unimi.it/dottorato_aab/)

(XXXIV cycle, 2018-20)

Project draft

1.Field of interest

AGR 03, AGR07

2.Project title

Genomics-based selection approaches for fruit quality traits in stone fruits

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4. Relevance of the topic and state of the art:

The use of genome-wide markers and high-throughput genotyping methods has led to the development of new strategies for analysis of complex quantitative traits, such as genome-wide association studies (GWAS) and genome-wide selection (GWS). GWAS have evolved over the last ten years into a powerful tool for investigating the genetic architecture of horticultural traits and Quantitative Trait Loci (QTL) identification. In the past years, a number of QTLs have been discovered for agronomically important traits in stone fruits, such as disease resistance and fruit traits, with relative high mapping resolution [1], [2], [3]. Molecular markers for these QTLs represent a useful resource for enhancing selection efficiency via marker-assisted selection (MAS) in breeding programs. However, while MAS is suitable for mono-/oligo-genic traits, ways to use loci with moderate to small effect are needed to further exploit the existing genetic variation and for polygenic traits. One of the most promising approaches is Genome-Wide Selection (GWS), which uses the entire genomic information to estimate future phenotypes or unobserved genetic/breeding values. Compared to MAS, GWS can better accommodate multiple-trait prediction models, when the selection objective is on several traits simultaneously. In GWS genotypic and phenotypic data are collected on a reference population to train a model for the estimation of individual breeding values (genomic-estimated breeding values, GEBV) which is then applied to select superior candidates based only on genotypic data [4], [5]. GWS represented a “paradigm shift” in livestock breeding and in recent years it has been extensively adopted and validated in a range of crop species [6]. Initial studies demonstrated the validity of this approach in fruit trees where GWS has been mostly used to predict fruit quality traits, as recently demonstrated in peach [7], as well other fruit tree species. In long-generation species such as fruit trees, the breeding cycle can be made significantly shorter by selecting young candidates based exclusively on genotypic information. However, there is no systematic work until now that used genome-wide approaches in genetic improvement for resistance traits in stone fruit species, or accounted for the interaction between genotype and environment (GxE).

5.Layout of the project (draft)

5.1.Materials & Methods

The PhD project is divided into three main activities: **phenotyping (T1), genotyping (T2) and data analysis (T3).**

T1: develop and apply phenotyping approaches for the screening of germplasm collection for fruit quality traits such as fresh and dry weight, soluble solid and metabolites (sugars, organic acids, polyphenols, aroma compounds) content, textural attributes. The aim is to collect data for GWAS and GWS studies. In apricot, a germplasm collection of more than

200 individuals with different origins, about 150 breeding selections from MASPES breeding program and several bi-parental crosses.. In peach, more than 300 among accessions and breeding selections will be available, along with several bi-parental crosses. In addition, a reference population (peach RefPop) consisting of 400 accessions (all grafted on the same rootstock) covering the genetic diversity of European peach germplasm and designed for GWAS and GWS studies will be also characterized.

T2. A combination of genotyping strategies will be applied to reach high-density genome-wide marker data for the different stone fruit species with minimal costs. Depending on the current status of genomic resources, different strategies will be considered, including SNP arrays in peach and genotyping-by-sequencing (GBS) in apricot. The 18K Illumina SNP array was developed from re-sequencing information for about 50 peach accessions as part of a collaboration between the EU FruitBreedomics and the US RosBreed projects. In the case of GBS/ddRAD, available and validated bioinformatics pipelines will be applied for SNP calling from raw data. Alternatively, a novel custom target SNP array approach will be developed in apricot, using whole-genome re-sequencing data of several accessions, freely available in publicly released databases.

T3. Genome-wide association studies will be performed, searching for association between genomic loci and resistance traits. In peach, the RefPop will be used in GWAS to dissect relevant phenotypes and pinpoint genomic regions underlying each trait. Results from re-sampled predictive models (i.e. replicated variable selection models for genome-wide predictions) will be matched with associations from GWAS, in order to obtain more robust signals of phenotype-genotype associations. For traits subjected to strong selective pressure, and for recessive traits, the analysis of runs of homozygosity (ROH) in the genome will further reduce the probability of finding spurious associations, and provide highly reliable associations. Genetic maps of progenies phenotyped under **T1** and genotyped under **T2** will be constructed and used to dissect the genetic control of fruit quality traits. Where possible, reference genome sequences will be used for candidate gene mining within QTL/MTL regions. For all traits, models for genome-wide prediction (GWP) will be developed, based on scope (e.g. within- or across-populations), traits (e.g. categorical, continuous, longitudinal), effects to be included alongside possible non-additive and non-linear relationships. Where available, records on the same plant in multiple years will be modeled natively by using an across-time covariance structure in a repeatability model [7]. The accuracy of genome-wide predictions will be measured through adequate cross-validation schemes, to avoid overfitting and ensure validity and reproducibility of results. Both the ability to predict breeding values, relevant for genetic improvement, and the ability to predict future phenotypes, relevant for plant management, will be estimated. Additionally, the heritability and number of genes involved in the determinism of each trait will be assessed, in order to provide relevant information to help design the most appropriate genome-assisted breeding scheme for each trait and population.

5.2.Schedule and major steps (3 years)

The previously described (paragraph 5.1) activities will be carried out following the above described plan:

T1 – year 1-2
T2 – year 1-2
T3 – year 2-3

6. Available funds (source and amount)

Genotyping will be carried out under a collaboration agreement with a private company which will take charge of these costs.
Orchard management and phenotyping activities will be supported by the MAS.PES breeding programme.

6. Literature

[1] T. Pascal *et al.*, “Mapping of new resistance (Vr2, Rm1) and ornamental (Di2, pl) Mendelian trait loci in peach,” *Euphytica*, vol. 213, no. 6, p. 132, 2017.

[2] S. Mariette *et al.*, “Genome- wide association links candidate genes to resistance to Plum Pox Virus in apricot (Prunus armeniaca),” *New Phytol.*, vol. 209, no. 2, pp. 773–784, 2016.

[3] J. A. Salazar, M.-A. Batnini, N. Trifi-Farah, D. Ruiz, P. Martínez-Gómez, and M. Rubio, “Quantitative trait loci (QTLs) identification and the transmission of resistance to powdery mildew in apricot,” *Euphytica*, vol. 211, no. 2, pp. 245–254, 2016.

[4] T. H. E. Meuwissen, B. J. Hayes, and M. E. Goddard, “Prediction of total genetic value using genome-wide dense marker maps,” *Genetics*, vol. 157, no. 4, pp. 1819–1829, 2001.

[5] A. J. Lorenz *et al.*, “Genomic Selection in Plant Breeding. Knowledge and Prospects.,” *Adv. Agron.*, vol. 110, no. C, pp. 77–123, 2011.

[6] Z. A. Desta and R. Ortiz, “Genomic selection: genome-wide prediction in plant improvement,” *Trends Plant Sci*, vol. 19, no. 9, pp. 592–601, 2014.

[7] F. Biscarini *et al.*, “Genome-enabled predictions for fruit weight and quality from repeated records in European peach progenies,” *BMC Genomics*, vol. 18, no. 1, p. 432, 2017.