IEF Final Report (625308 AlfalfaEvolution) - Summary

Summary description of the project objectives

The objectives of the proposed project were to: (i) determine the genetic structure within *Medicago sativa* (alfalfa) in order to characterize discrete wild genetic pools (populations); (ii) investigate the relationships among the distinct *M. sativa* populations and taxa; (iii) identify geographical origin of *M. sativa* intraspecific taxa and the forces driving their spread; and (iv) investigate ecological adaptation in wild populations in order to establish a basis for future breeding programs.

Description of the work performed since the beginning of the Project.

We requested seeds from the USDA-GRIN germplasm bank, germinated samples, obtained leaf samples and dried them in silica gel. We carried out a pilot study to check for variability in the genetic markers chosen. We performed flow cytometry for ploidy determination in all accessions. We performed fieldwork to sample additional populations. We extracted genomic DNA and prepared libraries for genotyping-by-sequencing (GBS) during a stay at a collaborating scientist's lab in the USA. Then we sent libraries for Illumina sequencing. We also performed bioinformatic routines to filter and process the data. We prepared additional libraries for gene capture of 11 different *Medicago* species closely related to *M. sativa*. We have performed preliminary analyses of the datasets obtained. We obtained 3 different genetic datasets ready to be analysed (*GBS-M. sativa: # of individuals used, GBS-M. sativa relatives: # individuals used* and *gene-cap M. sativa relatives: number of individuals used*).

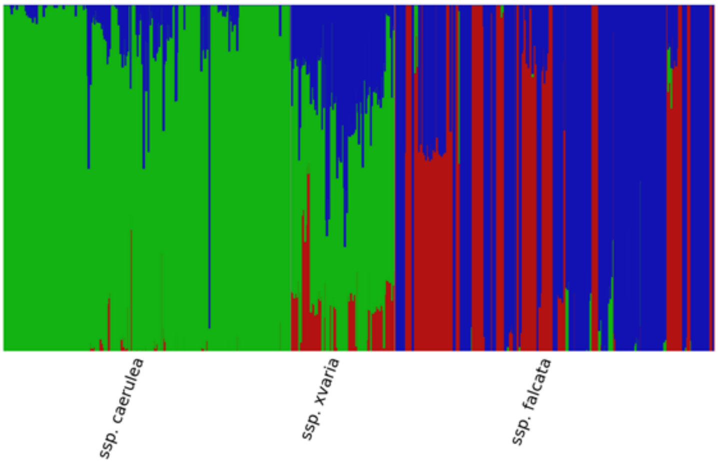

Fig. 1: Population structure of the diploid *M. sativa* subspecies indicating the presence of three genetic groups and a substantial proportion of admixed individuals.

Description of the main results achieved so far.

(1) We have performed analyses of population structure for the *GBS-M. sativa* dataset. Results show that the species is structured in 3 main clusters (Fig. 1). Subspecies *caerulea* forms a cluster, subspecies *falcata* is divided in two different clusters and subspecies *xvaria* is formed by admixed individuals with ancestry shared among the three clusters.

(2) We have also built a tree using the *GBS-M. sativa* dataset to show relationships among individuals (not shown). Low bootstrap support in the internal branches of the tree indicates that the species is not highly differentiated. Basal lineages in the tree included individuals found in central Asia, suggesting that this is the geographical origin of the species.

(3) For the *GBS-M. sativa relatives* dataset we generated a species tree (not shown). *M. sativa* was recovered as a natural group with *M. hybrida* as the closest relative.

(4) We are currently generating gene trees with the *gene-cap M. sativa relatives* dataset. These trees are highly incongruent among each other (not shown). This can be the result of extensive hybridization among the different species. Specific hybridisation analyses will be performed to confirm and detect the hybridisation events that have occurred in this group.

Conclusions and potential impact

We have just started to perform genome scans for selection in the *GBS-M. sativa* dataset and to investigate associations of genotypes with environmental variables. The results of these analyses will establish a basis for future successful breeding strategies of alfalfa cultivars. Alfalfa breeders will be able to use information about the genes that are targets of selection to increase survival of cultivars or to design breeding programs in the context of climate change. We will also investigate common patterns of adaptation between *M. sativa* and *M. truncatula*. This information will be highly relevant to determine how environmental adaptation occurs at different levels (populations and species), and to investigate if common strategies are found across different related species. This will be relevant for researchers investigating the genus *Medicago* and breeders of other *Medicago* species.

The study of the past colonization dynamics and hybridisation patterns in *M. sativa* will inform about how the species has responded to past climatic events and how strong were the reproductive barriers among species. This information will be also highly relevant to predict future range dynamics and transmission of genes among *Medicago sativa* and close relatives. Noteworthy are the signs that we have seen of extensive past hybridisation (among close relatives of *M. sativa*) and ongoing hybridisation (among *M. sativa* subspecies).

The role of hybridisation in plant evolution has been the subject of debate, with a destructive role (i.e., that hybridisation merges formerly distinct species and reduces biodiversity) contrasted with a creative role (i.e., that species receive genes through hybridisation and are thereby able to adapt to new niches). Our research provides a foundation for framing new and more specific questions regarding the role of specific hybridisation episodes between several species and subspecies of *Medicago* and *M. sativa*, respectively. The extent of ancient and ongoing hybridisation in this important crop species (*M. sativa*) and this genus as a whole had not been previously understood. We are now in a position to further develop this genus as a model system to understand the role hybridisation has played in adaptation to previous climate and other environmental changes, and use this knowledge to improve our understanding of how future climate change might affect plant species.