ABSTRACT

In the context of the breeding program carried out at IVIA, in this thesis we have developed tools for implementing the program and increase its efficiency. Being the genetic resources the main breeding tool, the genetic diversity of a germplasm collection has been studied using microsatellites molecular markers. We used Factorial Correspondence Analisys, Bayessian analysis and UPGMA cluster analysis to determine the population structure of the collection. We obtained 5 subpopulations related to the origin of the accession. Genetic distances and the grouping analysis suggest that accessions introduced in the Mediterranean Basin would come from Asia. On the other hand, we have obtained 3 subpopulations that include accessions from European origin which demonstrated the high varietal diversity and adaptability reached by this species although its late introduction in Europe. Additionally, the self-incompatible alleles provided valuable information about the germplasm movements and contributed to know the inter-compatibility groups into the collection. The genetic information gathered completes the phenotypic characterization made previously at IVIA, all together the results will be a valuable tool for planning the future crosses in the breeding program.

Another biotechnology tool developed for implementing the program was the techniques set up for increasing the diversity with new genotypes with different ploidy levels. We have applied chemical mutagenesis using colchicine and *in vitro* selection, aimed at obtaining polyploids, which are of high interest in loquat species, due to its potential for producing varieties with bigger fruits (tetraploids) o seedless fruits (triploids). We obtained stable polyploids soaking the seeds in a colchicine buffer. Two triploids (3*x*) were obtained, probably already present by natural mutation in the hybrid seed lot, and one tetraploid (4*x*). The ploidy level was determined by flow cytometry, the results were confirmed by chromosome counting in leaves and roots, and morphological analysis.

On the other hand, aimed at obtaining haploids and double haploids (DH), we studied the potential gametophytic embryogenesis induction in both gametes type, male (by isolated microspores culture and anthers) and female (*in situ* parthenogenesis induced by irradiated pollen). Homozygous lines obtained in a unique generation by biotechnology methods are very useful in long juvenile period species as loquat. The haploid genotypes allow obtaining of homozygous genotypes in one step, they simplify

genetic studies, allow alignment of sequences, and to exploit the hybrid vigor. The experiments made on isolated microspores succeeded for calli induction in several accessions used, which is the first morphogenetic step. The anther culture resulted in a triploid plant (3*x*) probably explained by a natural chromosome duplication during the regeneration process. Results demonstrated that induction of embryogenesis in loquat is possible; however it depends on many variables that need to be analyzed. Gynogenesis *in situ* by irradiated pollen with gamma rays and embryo rescue *in vitro* allowed obtaining four haploid plants. The ploidy level was determined by flow cytometry, the results were later confirmed by chromosome counting in leaves.