



## **Best CIHEAM Master Thesis**

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**Use of microsatellite markers (SSRs) to analyse the molecular variability and to identify the olive varieties of the Boughrara (Sfax, Tunisia) germplasm bank**

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## Abstract

Olive cultivation is one of the oldest activities developed by humans in the course of the History. As a consequence, most of the traditional olive producing countries have, nowadays, a diversified genetic patrimony which is mainly formed by autochthonous cultivars. Before the 90s of the previous century, no notable effort has been made to study olive cultivars in Tunisia. However, since the emergence of the modern oliviculture, the identification, the evaluation and conservation of the local genetic resources have been considered as a priority in countries like Tunisia.

In this work, a set of 8 previously-described microsatellite markers has been selected in order to analyse 84 olive accessions provided by the “Boughrara” (Sfax, Tunisia) Germoplasm Bank. The mentioned markers showed a good efficacy, since they displayed a high level of polymorphism, and no null allele presence was reported, except in the case of *ssrOeUA-DCA15*. The genetic diversity indexes generated in this report, like the total number of alleles (64), the number of alleles per locus (8), both the observed and the expected heterozygosity levels (averages of 0,77 and 0,68, respectively) and the polymorphic information content (average of 0,64), undoubtedly show that Tunisian olives, represented by this germplasm collection, possess a high degree of genetic diversity. Furthermore, 6 unique- and several low-frequency- alleles were detected, which can be considered markers of interest to separately identify several cultivars. The analysis of the probability of identity (cumulated probability:  $1,23 \times 10^{-7}$ ) allowed us to evaluate the discrimination power of the markers either used individually or in combination. The *UDO99-43*, *GAPU101*, *ssrOeUA-DCA16* and *ssrOeUA-DCA18* markers were able by themselves to discriminate a 82,5% of the whole genotypes identified by the 8 microsatellites markers all together.

The use of generic names by Tunisian olive growers to name cultivars, together with some mis-identifications, have generated the presence of synonymy and homonymy cases within the “Boughrara” (Sfax, Tunisia) Germoplasm Bank. According to the present study, only 47,6% of the analysed accessions represent unique genotypes (40 genotypes were identified among 84 accessions). In fact, the present study may provide support for a better management of the mentioned Germoplasm Bank and it will be useful to provide cultivar-guaranteed plant material to the currently rising plant nursery industry.

Finally, several statistic analyses have allowed us to establish the genetic relationships existing between the different genotypes identified, and also to perform a factorial analysis of correspondence to characterize clustering tendencies. The analysis of the genetic relationships showed the presence of 4 cladistic groups with a good level of internal relationship, as well as 5 genetically separated cultivars, probably as a consequence of their different origins, which contained unique or low frequency alleles. On the other hand, the existence of a small intra-cultivar variation was detected, possibly due to the presence of somatic mutations, particularly in some widely spread cultivars like ‘Chemlali Sfax’.

The correspondence factorial analysis has shown the appearance of a tendency of genetic separation clearly revealed among the cultivars destined to olive table consumption. This genetic drift is most likely a consequence of the prolonged selection of different genotypes according to farmers’ needs throughout olive History in Tunisia. However, no genetic separation has been reported on the basis of the geographical origin of the different cultivars, perhaps as the result of the geographical continuity of the olive-growing regions of the country, which has contributed to the genetic interchange.

The morphological characteristics of the fruit endocarps have also been analyzed in detail. These characteristics confirm the data obtained after the analysis of SSR markers, especially as regards to the wide variability in the collection, the existence of synonyms and homonyms, and the genetic separation based on cultivars uses (table/oil).