Abstract

Microspore-derived embryogenesis is the most common androgenic route to obtain doubled haploid (DH) individuals, 100% homozygous, which can be used as pure lines. This process is a biotechnological alternative to the classic breeding programs that allows for a reduction in the time and the resources needed to produce commercial hybrids. Due to the advances made in the last 30 years, some species can be considered as model organisms because of their high efficiency to produce DHs. However, there are still many species, interesting from a commercial and agronomical point of view, which remain recalcitrant to androgenesis induction. The biotechnological potential of this technique makes it essential to improve this process in those species where androgenesis is not yet optimized. For this, it is necessary to combine both applied and basic approaches, trying on the one hand to find the best experimental conditions, and on the other hand, exploring the basis of the reprogramming of microspores, in order to increase the possibilities of influencing the process. In this Thesis we have used both approaches, using rapeseed as model species and pepper as a recalcitrant species. In pepper, the study of key factors for androgenesis induction allowed us to optimize an anther culture protocol that can be applied to different genotypes. We propose the combination of calyx/bud ratio (80-90% of the bud covered by sepals) together with anther pigmentation (anthers with purple apical end) as reliable and easy-to-measure morphological markers to determine which buds and anthers contain the microspores at their optimal stage to induce androgenesis. We also demonstrated that the presence of somatic calli is more dependent on the culture conditions than on the genotype. This, in turn, allowed us to obtain a protocol that reduces the presence of somatic calli and increases the number of embryos obtained.

In the basic research studies made with rapeseed, the use of high pressure freezing followed by cryosubstitution allowed us to observe the ultrastructural changes undergone by the induced microspores. We studied the architecture and composition of the newly-formed cell wall in embryogenic microspores. We observed that these cell walls were deformed and incomplete, which could contribute to the well known phenomenon of nuclear fusion, which in turn leads to genome doubling, typical of DHs. We proved that these cell walls presented high levels of callose and absence of cellulose. We also studied the architecture of different organelles present in just induced microspores. We showed that plastids of embryogenic microspores behave as autophagic plastids (plastolysomes). These ultrastructural changes allowed us to define two new androgenic markers: the presence of callose in the first newly-formed cell walls and developing cell plates, and the presence of plastolysomes probably acting as part of a cytoplasmic cleaning mechanism, in parallel to microspore reprogramming.