

Abstract

Androgenesis is an inducible process that allows to obtain doubled haploid, pure lines through embryogenesis or callogenesis, starting from male gametophytes (young bicellular pollen) or their precursors, the microspores. From a biotechnological perspective, this possibility has become very relevant because pure lines are the base of hybrid seed production, and doubled haploid technology reduces the 8 or 9 generations necessary obtain a pure line with the conventional selfing and selection approaches, to only one generation.

The present Dissertation focuses on the study of androgenesis applying in parallel two approaches: (1) a basic one, related to the study of the factors that influence this process and the changes undergone by the microspores when induced; and (2) an applied approach directed to improve induction efficiency in recalcitrant species. For this work, we aimed three plant species: rapeseed (*Brassica napus*), used as a model plant for the study of androgenesis, and two species described as recalcitrant, tomato (*Solanum lycopersicum*), and eggplant (*Solanum melongena*).

We used High Pressure Freezing (HPF) and Freeze substitution (FS) to process rapeseed isolated microspore cultures to study the ultrastructural changes undergone in the induced microspores. We also optimized a system for genetic transformation of microspores, and further induction of secondary embryogenesis over the microspore-derived primary embryos, with the purpose of using this system as a tool for basic studies.

In tomato, we have characterized the process of androgenesis induction through anther culture. We demonstrated that the meiocyte is the inducible stage, instead of the microspore, which is the most sensitive stage in many other androgenic systems. Upon induction, meiocytes produce calli, coming most of them from somatic cells or from the fusion of two haploid nuclei of the meiocyte. These observations could explain the difficulty to obtain doubled haploids from tomato anther cultures.

In eggplant we developed an efficient system for the production of double haploid plants through isolated microspore culture. In this case, doubled haploid plants were obtained through callogenesis and further organogenesis. We also investigated the role of several factors, previously used in other plant species, in the process of induction of the eggplant microspore. Most of the tested factors showed a positive effect in the androgenic response, in growth and in the quality of the material obtained.

In summary, the studies presented in this work help to increase our knowledge about the androgenic process through its study in both model and recalcitrant species. Besides, they allowed us to present a new and efficient method to obtain doubled haploids in eggplant through microspore culture.