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EVOLUTION OF TISSUE CULTURE ON CANNABIS SATIVA THROUGH THE USE OF ASSISTED TECHNOLOGIES: A PROMISING APPROACH FOR THE DEVELOPMENT OF AGRICULTURAL BIOTECHNOLOGIES

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The species *Cannabis sativa* (L.), due to its genetic variability and plasticity, can produce a myriad of bioactive compounds with high therapeutic and nutraceutical value. This has allowed rapid development of this market in recent years, although there are several legal constraints that still limit the use of this plant to only a few varieties and applications. Despite its potential economic value, there is still much to be investigated on the genetic aspects and biology of this crop, to fully understand the molecular mechanisms regulating its key traits. One way to achieve this is using innovative technologies, such as tissue culture and biotechnology for genetic improvement. Micropropagation is a technique that involves the propagation and maintenance of plants under controlled, sterile conditions; it allows the cloning of plants with desirable traits, reducing maintenance space, costs and ensuring a contamination-free product. Tissue culture involves the growth of plant cells and their differentiation in a controlled environment. This allows, in addition to the application of precise targeted genetic modifications, the possibility of restoring plant material affected by viruses and viroids such as the HLVd (Hop Latent Viroid), a major problem in the Cannabis agricultural and plant breeding sector. Furthermore, it’s a *conditio sine qua non* for functional studies *in planta*. *C. sativa* is considered a recalcitrant

species for *in vitro* culture; at the Research Center for Cereal Crops and Industrial Crops of CREA, Bologna site, we are developing a new micropropagation medium for in vitro maintenance, multiplication and rooting of *C. sativa* explants. We focused on balancing the macro and micro nutritional elements differently from the soils usually used for C. sativa for a better shoot development. Next, we focused on regeneration protocols from different types of explants. Indeed, regeneration it’s a critical bottleneck for further development of biotechnology in this species, with published protocols reported a very low frequency of success, and their reproducibility in most cases has not yet been demonstrated. To date, we have verified the success of regeneration protocols (caulogenesis) from hypocotyls and cotyledons of three varieties bred by CREA, while no regeneration from mature explants taken from micropropagated shoots has been achieved yet. The poster will outline the main results obtained to date and future goals. This work is conducted as part of the Ph.D. program, in Agribusiness Science, Technology and Biotechnology, entitled "Innovation and breeding in medical Cannabis" of the University of Modena and Reggio Emilia. The goal is to increase the scientific knowledge and progress in the genetic improvement of Cannabis, developing innovative biotechnological tools and protocols that will allow researchers to study and modulate the function of target genes for the development of new Cannabis ideotypes.