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PLANT CELL CULTURES FROM *JATROPHA CURCAS*: A POSSIBLE SOURCE OF RENEWABLE ENERGY

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Negative environmental consequences of fossil fuels and concerns about petroleum supplies have spurred the search for renewable transportation biofuels. Biodiesel is the most used alternative biofuel nowadays, along with bioethanol, in the world. The increasing demand of plant species producing oil rich seeds is leading to the reduction of agricultural areas assigned to crops grown for human nutrition and it is causing food price raise. *Jatropha curcas,* a plant native of Mexico and Central America, has attracted the interest due to its easy adaptability to semi-arid marginal sites, its seeds producing non-edible oil used as a diesel fuel substitute and its role in erosion control. Considering its enormous potential, a large amount of quality planting material is required in the future. The aim of the proposed project is to design reliable protocols to obtain biomass production and to induce lipid accumulation in cell cultures obtained from *J. curcas* explants. The experimental plan led to the establishment of protocols able to stimulate cells proliferation from leaf and seed explants and to the set up of easy and fast clonal propagation by cuttings, organogenesis and somatic embryogenesis. Cell proliferation was obtained in many different conditions of salt and phytohormone combinations in the culture medium. *In vivo* propagation through cuttings was carried out from plants of different age and origin. First results indicate that the hardwood part of the plants is the most efficient in rooting and outliving. Organogenesis via a callus-mediated step and plant regeneration were achieved *in vitro*, but improvements are needed both in propagation techniques and adaptation to natural environment modalities. Some protocols to induce repetitive somatic embryogenesis in *J. curcas* have been applied starting from different explants (leaf, hypocotyl, seed).

Genetic engineering through transformation is a valuable method for the development of oil enriched varieties. Susceptibility of *J. curcas* leaf explants to *Agrobacterium*-mediated transformation was tested, and transformed calli constitutively expressing GFP protein are now available in our laboratory. On this basis, *Agrobacterium*-mediated transformation was planned to insert a gene involved in the plant lipid biosynthesis pathway. The chosen gene encodes for an enzyme, diacylglycerol acyltransferase (DGAT), that catalyzes the final rate-limiting step of the triglycerides condensation. A cloning cassette with *A. thaliana* DGAT gene fused with the eYFP gene, under the control of a constitutive CaMV 35S promoter, has been cloned into a pGreen-based delivery plasmid. *Agrobacterium*-mediated transient transformations are being performed to test the correct expression of the fusion protein.